

**Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development**

Birth Cohort Studies

Manual of Procedures II

For children 37-60 Months of age

Contents

[**Introduction** 3](#_Toc428278917)

[**QCS—Surveillance Quality Monitoring: Guidelines for Field** **Supervisors** 5](#_Toc428278918)

[**Extension Protocol Re-Enrollment** 8](#_Toc428278919)

[**ICF – Intervening Conditions Form** 9](#_Toc428278920)

[**SLF – Schooling Form** 11](#_Toc428278921)

[**Household Surveillance** 13](#_Toc428278922)

**E**[**NC—Extension Non-Continuation Form SOP** 14](#_Toc428278923)

[**PDF—Protocol Deviation Form SOP** 15](#_Toc428278924)

[**AEF—Adverse Event Recording and Reporting SOP** 17](#_Toc428278925)

[**REF—Child Referral Form SOP** 19](#_Toc428278926)

[**MOD—Monthly Form** 22](#_Toc428278927)

[**AVF – Anthropometry and Vaccine Form** 25](#_Toc428278928)

[**ANT – Anthropometry Check Form** 27](#_Toc428278929)

[**Dietary Assessments** 30](#_Toc428278930)

[**NUT—Nutrition Form** 31](#_Toc428278931)

[**FRC.FRE SOP – Food Recall Forms** 34](#_Toc428278932)

[**Cognitive Assessments** 38](#_Toc428278933)

[**WPPSI—Wechsler Preschool and Primary Scale of Intelligence - III** 39](#_Toc428278934)

[**EFT – Executive Functioning Task** 45](#_Toc428278935)

[**MAB—Movement Assessment Battery for Children-2** 28](#_Toc428278936)

[**SDQ—Strengths and Difficulties Questionnaire** 33](#_Toc428278937)

[**HOM—HOME Inventory (Early Childhood)** 39](#_Toc428278938)

[**SRQ—Self-Reporting Questionnaire 20** 51](#_Toc428278939)

[**Collection and Processing of Clinical Specimens** 53](#_Toc428278940)

[**USV— Small Volume Urine Collection, Processing, and Transport** 54](#_Toc428278941)

[**BCH—Blood Collection SOP** 56](#_Toc428278942)

[**HEM—HemoCue Assay SOP** 63](#_Toc428278943)

[**BRF—Blood Processing, Transport and Storage and Blood Receiving**  67](#_Toc428278944)

[**SFC—Stool Collection, Processing and Transport to the Laboratory** 70](#_Toc428278945)

[**SRF—Receiving and Storage of Stool Samples SOP** 73](#_Toc428278946)

[**MDF—Microscopy for the Detection of Ova and Parasites in Stool** 76](#_Toc428278947)

[**QIAamp Fast DNA Stool Mini Kit SOP** 80](#_Toc428278948)

[**SES & Food Security** 85](#_Toc428278949)

[**FSE—Followup SES Questionnaire** 86](#_Toc428278950)

[**FSQ—Food Security Questionnaire** 97](#_Toc428278951)

**Introduction**

This Manual of Procedures is the source document for the Standard Operating Procedures (SOPs) to be used for the extension of the MAL-ED project “Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development”. Specifically, the extension study is intended to acquire data from children enrolled in the original birth cohorts at each of the eight MAL-ED sites on their cognitive development and the extent of catch-up growth or of further growth faltering and the kinetics of their immune response at five years of age.

It is recognized that during the period of time since the completion of the 0-24 month study (at end of February, 2014) sites have continued different degrees of contact and data collection with the cohorts without funding. With the availability of new funds we will continue to follow as many enrollees as possible using a minimal common protocol that will permit multi-site data analysis at the completion of the study in 2017.

**Table 1. Timing of Assessments and Measurements used for the MAL-ED Extension Study**

|  |  |  |
| --- | --- | --- |
| **Assessment** | **Timing** | **NOTES, measurement information** |
| **Surveillance** | **Monthly questionnaire** | MOD: To gather health status information and maintain contact with cohort children |
| **Cognitive Assessment** | **At 60 months of age** | *Cognitive Skills -*The Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III)  *Executive Function (EF)–*Day Night Stroop; Dimensional Change Card Sort; Forward Word Span; Go-No Go; Forward Corsi Block Tapping  *Motor Skills -*Movement Assessment Battery for Children (MABC, 2)  *Socioemotional Skills -*Strengths and Difficulties Questionnaire (SDQ) |
| **Anthropometry and vaccine information** | **Quarterly** | AVF: Height and weight and vaccines |
| **Nutrition survey** | **At 60 months of age** | Nutrition (NUT) Questionnaire (optional monthly 37-60m)  3 FRC (60 months)  FRE (60 months) |
| **Blood** | **At 60 months of age** | BCH, BRF: Archive for future micronutrient testing (to be related to cognitive development  assessments) and immunoassays |
| **Urine** | **At 60 months of age** | USV: Archive for future iodine testing (to be related with cognitive development assessments) |
| **Stool** | **At 60 months of age** | SFC/SRF: Collect asymptomatic (monthly) stool, perform microscopy (MDF) and archive for TACMAN assay |
| **SES questionnaire** | **At 60 months of age** | FSE, FSQ: To control for changes in SES |
| **Maternal and environmental assessment** | **At 60 months of age** | HOM, SRQ: Home and SRQ instruments (instruments used in original protocol but a different version of the HOME will be used  at 60 months) |

**QCS—Surveillance Quality Monitoring: Guidelines for Field** **Supervisors**

**I. Purpose**

To provide general guidelines and minimum standards for surveillance data collection quality control activities by the field supervisor.

**II. Methods**

The field supervisor will supervise a team of Study Researchers / Nurses / Fieldworkers / Data Collectors who will administer all of the surveillance forms at the appropriate times. The field supervisor should use locally appropriate management techniques in order to ensure complete, accurate and timely data collection.

The field supervisor should monitor:

1. Training
   * The supervisor should ensure that all staff is appropriately trained to collect the data.
   * The supervisor should be aware of changes to forms and procedures and ensure timely retraining of staff (within one week of announcement of changes).
   * The supervisor should review all forms collected by staff and identify inconsistencies or mistakes that indicate retraining is necessary.
   * The supervisor should hold periodic (at least quarterly) refresher training sessions with a focus on data collection topics identified as problem areas. For example, if retention is becoming a problem, the supervisor should organize a training session on how to keep participants in the study. During that training session, staff can brainstorm about retention methods or (no- or very low-cost) incentives that might be offered in order to increase retention, or the supervisor could bring in an expert from the local university to discuss these issues.
2. Data collection

Ideal collection windows for key forms/samples

|  |  |  |  |
| --- | --- | --- | --- |
| **Forms** | **Form name** | **Month(s)** | **Visit range** |
| AVF | Anthropometry and Vaccine Form | 39, 42, 45, 48, 51, 54, 57, 60 | DOB (+/-) 7 DAYS |
| BCH/BRF | Blood collection | 60 | DOB - 2/+12 DAYS |
| EFT | Executive functioning | 60 | DOB (+/-) 30 DAYS |
| FRC/FRE | 24 hour food recall | 60 (3 visits within a 4 week period) | DOB -30 /+15 DAYS |
| FSE | Follow-up SES | 60 | DOB (+/-) 15 DAYS |
| FSQ | Food security questionnaire | 60 (optional) | DOB (+/-) 15 DAYS |
| HOM | HOME | 60 | DOB (+/-) 30 DAYS |
| ICF | Intervening Conditions form | Administered one time upon re-enrollment | (+/-) 15 DAYS |
| MAB | Movement assessment battery | 60 | DOB (+/-) 30 DAYS |
| MOD | Monthly form | 37-60 | DOB (+/-) 2 DAYS |
| NUT | Nutrition form | Monthly assessment from month 37-60  (optional) | DOB -2/+7 DAYS |
| SDQ | Strength and difficulties questionnaire | 60 | DOB (+/-) 30 DAYS |
| SFC/SRF | Stool collection | 60 |  |
| SRQ | SRQ-20 | 60 | DOB (+/-) 30 DAYS |
| SLF | Schooling form | Administered one time upon re-enrollment | (+/-) 15 DAYS |
| USV | Urine collection form | 60 | DOB -2/+7 DAYS |
| WSI | Wechsler Preschool and Primary Scale of Intelligence - III | 60 | DOB (+/-) 30 DAYS |

* + Organize schedules for Study Researchers / Nurses / Fieldworkers in order to ensure all data are collected on each participant in a timely manner (see schedule of forms/sample collection above). Data should be collected within the collection windows identified for each form, and if data are collected outside the window, a PDF should be filled out.
  + Review 100% of each Study Researcher / Nurse / Fieldworker’s forms at least on a weekly basis, ideally, at the end of each working day. Supervisors should ensure the forms are complete (no missing fields), that the data appear correct, and that visits planned for the day / week were made.
  + Corrections to the forms should be minimized (data should be correct the first time), but if needed, the supervisor should ensure that the Study Researcher / Nurse / Fieldworker follows proper correction procedures. If form corrections are necessary, the data collector should:
    1. Cross through the incorrect information with one line only,
    2. Write the correct information,
    3. Write the date the correction was made, and
    4. Write their initials.
  + Make sure forms are kept confidential and protected (in a locked file drawer, for example) when they are returned from the field.
  + Specific quality control plans have been developed for anthropometry. Apart from the general quality control methods listed above (supervisor review of 100% of forms at the end of each day) there are no specific quality control plans for other forms.
  + Supervisors should keep up-to-date on changes in the SOPs and CRFs and retrain staff as needed. Frequent meetings, preferably weekly, should be held with the data collection staff in order to provide form or procedural updates to the staff, talk about any issues that have been observed, and in general, touch base with the staff and identify any questions or training needs.
  + Real-time check of anthropometry – In order to correct errors in anthropometry measurement in a timely manner, supervisors (or field workers) are encouraged to print out growth charts and draw points on the chart for each measurement. If the point is wildly inconsistent with the previous points (decrease of more than 2 centimeters in height, gain/loss of approximately more than 1 z-score of height or weight), prompt remeasurement (within 3 days of original measurement) is recommended. Growth charts can be found in the forms appendix and here: <http://www.who.int/childgrowth/standards/en/>. If remeasurement occurs and the original value was approximately correct, it should remain as originally written. If the original value was grossly inaccurate (>10% difference), replace with the more accurate value as long as the remeasurement occurs within one week (preferably sooner) of the original measurement. Follow standard cross-out procedures, and add a note with the date that remeasurement occurred to the form. If the child is very underweight or stunted, please follow your site-specific guidelines for managing/referring these children.

- Data center transmission of forms - Once forms have been completely filled out by the Study Researchers / Nurses / Fieldworkers and reviewed / initialed by the supervisor, they must be delivered within five days to the local data center for data entry. Data entry of the forms should occur within one month of delivery to the data center (preferably sooner).

1. Database review

After the data are entered, reports will be generated by the DCC so that supervisors can review the collected data for inconsistencies and errors. (e.g., With your knowledge of the expected diarrhea rates in the area, are the numbers what you expect them to be? Are the diarrhea rates similar across Study Researchers / Nurses / Fieldworkers? etc.) Quality control reports can be generated more frequently at the local level if desired.

**Extension Protocol Re-Enrollment**

**ICF – Intervening Conditions Form**

1. **Purpose**

The Intervening Conditions Form (ICF) is administered to all MAL-ED children once upon re-enrollment to MAL-ED extension study. The purpose of this form is to capture illness-related information from the time that the child was last visited for the MAL-ED study and the time of re-enrollment in the MAL-ED extension study. If a site has already been capturing this information on a monthly basis, there is no need to administer the ICF, as this data will already be known to the sites. Please use this data to complete the ICF for each child in the study.

1. **Materials**

Intervening Conditions Form, pen, clipboard

1. **Methods**

At time of re-enrollment, the fieldworker will complete the ICF.

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| 01 | Study Researcher/Nurse/Fieldworker ID | ### |
| 02 | Date | DD/MMM/YY |
| 03 | Date of Last Visit | DD/MMM/YY |
| **HEALTH** | | |
| 04 | Since the last time the MAL-ED team visited your child, has your child been hospitalized? | Yes = 01  No = 00 |
| If yes, specify the number of times  **If no, skip to #06** | If child was hospitalized, enter number of times child was hospitalized (precede any number less than 10 with a 0; for instance, if the child has been hospitalized for 2 days, enter 02). |
| 05 | If yes, what was the diagnosis/illness  (write diagnosis) | Character limit is 255 characters. |
| 06 | Has the child had any serious illness for which they were not hospitalized since the MAL-ED team last visited?  **If no skip to #08** | Yes = 01  No = 00 |
| 07 | If yes, what was the diagnosis/illness  (write diagnosis) | Character limit is 255 characters. |
| 08 | Has the child had any serious accidents/injuries since the MAL-ED team last visited?  **If no skip to #10** | Yes = 01  No = 00 |
| 09 | If yes, what was the accident/injury? (describe accident/injury) | Character limit is 255 characters. |
| **DEVELOPMENT** | | |
| 10 | Since the last time the MAL-ED team visited your child, did you notice your child having any difficulties with: | Vision=01  Hearing=02  Ability to communicate (i.e., using gestures)=03  Ability to speak words others can understand=04  Ability to understand others' spoken words=05  Coordination with walking or running=06  Coordination using their hands or fingers=07  Hand-eye coordination (e.g., catching a ball)=08  Muscle strength=09  Bladder control=10  Bowel control=11  Seizures=12  Head trauma=13  No difficulties noticed=14 |

**SLF – Schooling Form**

1. **Purpose**

The Schooling form (SLF) is administered to all MAL-ED children once upon re-enrollment to MAL-ED extension study. The purpose of this form is to capture schooling information from the time that the child was last visited for the MAL-ED study and the time of re-enrollment in the MAL-ED extension study.

1. **Materials**

Schooling Form, pen, clipboard

1. **Methods**

The fieldworker will ask the questions to the mother/caregiver for the child. For questions asking about number of months in school, please use appropriate rounding techniques.

|  |  |  |  |
| --- | --- | --- | --- |
| **#** | **Question** | **Guidance** | **Explanation** |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |  |
| 01 | Study Researcher/Nurse/Fieldworker ID | ### |  |
| 02 | Date | DD/MMM/YY |  |
| **SCHOOLING** | | |  |
| 03 | Has your child ever been to any type of school? | Yes = 01  No = 00 |  |
| 04 | How many months (in total) in daycare? | Write in number of months or use NA= did not attend daycare | Daycare refers to a setting where the child is being looked after but no formal school training is occurring |
| 05 | How many months (in total) in preschool? | Write in number of months or use NA= did not attend preschool | Preschool refers to a setting where the child has not yet entered FORMAL schooling but the child is likely to have some structured training during the day (examples include the caregiver/teacher reading to the children, doing crafts with the children, coloring with the children, etc.) |
| 06 | How many months (in total) in kindergarten? | Write in number of months or use NA= did not attend kindergarten | Kindergarten refers to a setting where the child is now in school with a teacher and the teacher has the responsibility of formally teaching the child. In some sites, kindergarten is required while in others it is not. It typically occurs the year before the child enters primary school. |
| 07 | How many months (in total) in primary school? | Write in number of months or use NA= did not attend primary school | Primary school refers to a setting where the child is receiving a formal education by a trained teacher. |
| **LANGUAGE**  (fieldworker should complete if able or ask caregiver if unsure) | | |  |
| 08 | What is the primary language spoken by child | Bengali=01  Tamil=02  Nepali=03  Newari=04  Sindhi=05  Portuguese=06  Spanish=07  Tshivenda=08  Swahili=09  Iraqw=10  Other=11 |  |
| 09 | If other, list: |  | Character limit is 255 characters. |
| 10 | What is the primary language heard by child? | Bengali=01  Tamil=02  Nepali=03  Newari=04  Sindhi=05  Portuguese=06  Spanish=07  Tshivenda=08  Swahili=09  Iraqw=10  Other=11 |  |
| 11 | If other, list: |  | Character limit is 255 characters. |

**Household Surveillance**

**ENC—Extension Non-Continuation Form SOP**

**I. Purpose**

To collect information about the reason a study participant is lost to follow up.

**II. Materials**

Extension Non-Continuation Form (ENC), ENC SOP, pen, clipboard

**III. Methods**

The Study Researcher / Nurse / Fieldworker will fill out this form if:

- the primary caretaker would like to withdraw the child from the study, or

- the child’s family decides to move out of the surveillance area, or

- the child dies during follow up, or

- the child is 37-60 months of age and the primary caretaker is not available to answer questions for more than 6 months and their whereabouts are unknown.

The supervisor is encouraged to identify specific reasons for dropping out of the study and to put into place measures to minimize additional drop outs.

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
|  | Participant ID | Write the participant ID in the space provided at the upper left corner. |
| 01 | Study Researcher / Nurse / Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker ID number here. |
| 02 | Today’s date | Format DD/MMM/YY |
| 03 | Date of extension re-entry | Format DD/MMM/YY |
| 04 | Reason for leaving the study | Document the reason for leaving the study here.  Dropped out=00: If the subject is being withdrawn from the study, enter 00 and probe for reasons why they do not want to participate. Write any reasons they give for dropping out in the notes section of the form.  Moved=01 : If they are moving, determine if they are moving out of the study area – If they move within the study area, we can continue to follow them. If they are moving out of the study area, enter 01.  Dead=02: If the subject dies, enter 02.  Unknown=03 : If the both the child and the primary caretaker have not been available for 6 months and you cannot get information about where they are, enter 03. |
| 05 | Date of last contact | Enter the date of last contact with the study subject. Format DD/MMM/YY. |
|  | Notes | Enter notes here, if relevant. Notes can include: reasons for leaving the study, new address if available, cause of death, any additional information. |

**PDF—Protocol Deviation Form SOP**

**I. Purpose**

To record deviations from the MAL-ED standard protocols.

**II. Material**

Protocol Deviation Form, pen.

**III. Methods**

The Principal Local Investigator is notified immediately when an event occurs outside of the standards of practice (this event is called a **protocol deviation,** which is any unapproved change, deviation, or departure from the study design or procedures). A Protocol Deviation Form is completed by the individual having committed the deviation and the study coordinator/sponsor/IRBs are notified. These documents should be retained at the site in a folder labeled (Protocol Deviations). They will be narrative in form, detailing what was done differently than called for in the protocol and why. Both the person committing the protocol deviation and the Supervisor should sign and date the Protocol Deviation Form.

An example of a protocol deviation is if a data form is collected outside the ideal date range (the acceptable window period). If this occurs, a PDF should be filled out. See the specific form SOP for exceptions to this rule.

Ideal collection windows for key forms/samples:

|  |  |  |  |
| --- | --- | --- | --- |
| **Forms** | **Form name** | **Month(s)** | **Visit range** |
| AVF | Anthropometry and Vaccine Form | 39, 42, 45, 48, 51, 54, 57, 60 | DOB (+/-) 7 DAYS |
| BCH | Blood collection | 60 | DOB - 2/+12 DAYS |
| EFT | Executive functioning | 60 | DOB (+/-) 30 DAYS |
| FRC/FRE | 24 hour food recall | 60 (3 visits within a 4 week period) | DOB -30 /+15 DAYS |
| FSE | Follow-up SES | 60 | DOB (+/-) 15 DAYS |
| FSQ | Food security questionnaire | 60 (optional) | DOB (+/-) 15 DAYS |
| HOM | HOME | 60 | DOB (+/-) 30 DAYS |
| ICF | Intervening Conditions form | Administered one time upon re-enrollment | (+/-) 15 DAYS |
| MAB | Movement assessment battery | 60 | DOB (+/-) 30 DAYS |
| MOD | Monthly form | 37-60 | DOB (+/-) 2 DAYS |
| NUT | Nutrition form | Monthly assessment from month 37-60  (optional) | DOB -2/+7 DAYS |
| SDQ | Strength and difficulties questionnaire | 60 | DOB (+/-) 30 DAYS |
| SRQ | SRQ-20 | 60 | DOB (+/-) 30 DAYS |
| SLF | Schooling form | Administered one time upon re-enrollment | (+/-) 15 DAYS |
| USV | Urine collection form | 60 | DOB -2/+7 DAYS |
| WSI | Wechsler Preschool and Primary Scale of Intelligence - III | 60 | DOB (+/-) 30 DAYS |

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
| 1 | Fieldworker ID/Lab technician ID | Enter the fieldworker or lab technician’s unique ID number here. |
| 2 | Today’s date | Enter the today’s date. Format DD/MMM/YY |
| 3 | What is the letter ID of the affected SOP? | Enter the SOP ID code here. Enter the primary SOP that is affected by the deviation. If additional SOPs are affected, you may note them in the Observation section at the bottom of the form. |
| 4 | Date of deviation | Enter the date the protocol deviation occurred. Format DD/MMM/YY |
| 5 | Participant ID of the affected subject | Enter the ID of the affected subject |
| 6 | Sample ID of the affected subject | Enter the sample ID here. If the protocol deviation does not apply to a specimen enter NA. |
| 7 | Did the deviation result in an adverse event? | Enter 00 for no; enter 01 for yes. If yes, complete adverse event form. |
| 8 | Reason for deviation | Enter the reason for the deviation. Possible answers include: Subject illness=01; Subject unable to comply=02; Subject refusal=03; Fieldworker error=04; Specimen leaked, test not performed=05; Specimen not labeled, test not performed=06; Clerical error (incorrect PID, SID or test result)=07; Test failure (technical)=08; Investigator/study decision=09; Other=10 |
| 9 | Observation/Notes | Describe the circumstances, reasons for, and implications for the study, if any, of the protocol deviation |

**AEF—Adverse Event Recording and Reporting SOP**

**I. Purpose**

To document and report adverse events that may occur to participants during the course of the MAL-ED study.

**II. Materials**

Adverse Events Form (AEF), pen

**III. Methods**

During the course of this study, adverse events (AE’s) will almost certainly occur that involve study subjects (enrolled child, mother, or other care givers). Most of these events will be minor, but some may be more serious and even life threatening. Whether minor or serious, they may impact our ability to obtain data and interpret it correctly. It is, therefore, important to record and report these events as they occur. As MAL-ED is not an interventional study involving experimental treatments or drugs, we do not anticipate that serious events will be related to the study procedures we will employ. However it is possible that serious illness or accidents will occur amongst our enrollees that result in serious injury or even death and will impact their continued participation in the study. These events should be recorded and analyzed by site investigators, including the site PI, to evaluate the relatedness of the event to our study protocol and its likely or real impact on our results. Adverse events, especially serious ones, may be reportable to your local IRB as dictated by site specific protocols.

An example of AE’s that may be study related include: bruising at the site of blood draw. Both the person completing the AEF and the supervisor should sign the form. Sites will maintain a file labeled “Adverse Events” where all AEF forms will be kept. These forms will not be uploaded to the central database but you may wish to enter them into your own database (at the discretion of the site PI). An AEF form should be completed by staff and the site PI or supervisor notified immediately. In those instances where an AE is determined to be possibly related to a study procedure, as well as all SAE’s whether study related or not, a scanned copy of the AEF should be sent by email (or FAX if scanning is not possible) should be sent to Dennis Lang (Lang4@fnih.org) or Karen Tountas ([ktountas@fnih.org](mailto:ktountas@fnih.org)) for our records. We will look for any trends across sites and determine if we need to adjust any study procedure as a result.

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
| 01 | Study researcher / Nurse / Fieldworker ID | Enter the fieldworker or lab technician’s unique ID number here. |
| 02 | Today’s date | Enter the today’s date. Format DD/MMM/YY |
| 03 | Did the adverse event result in death | Enter 00 for no; enter 01 for yes. |
| 04 | Date of adverse event | Enter the date of the event. Format DD/MMM/YY |
| 05 | Date of event resolved | Enter the date of the resolution if applicable. Format DD/MMM/YY |
| 06 | Is the adverse event possibly study related? | Enter 00 for no; enter 01 for yes. |
|  | Description | Provide details on the diagnosis, medical management; attach additional page if needed). If answer to #6 above is yes, explain why you believe the event may be study related |

**REF—Child Referral Form SOP**

**I. Purpose**

To document child illness referrals and outcome of those referrals.

**II. Material**

Child Referral Form (REF), pen, clipboard

**III. Methods**

1. This form is filled out as a follow up to referrals made by the Study Researcher / Nurse / Fieldworker, to document self-referral to medical care, and to document medication taken without contact with the medical system. It is important for the study to document medical care and treatment received by the child in order to have a good picture of the health of the child.
2. If the child is hospitalized or the Study Researcher / Nurse / Fieldworker is unable to get information about a diagnosis, the Study Researcher / Nurse / Fieldworker should wait until the child is discharged or until the information is available to fill out questions 9-14.
3. If the child becomes seriously ill or is hospitalized, the Principal Investigator must be notified.
4. When filling out this form, the Study Researcher / Nurse / Fieldworker should request to see the documentation from the healthcare provider. This can help the Study Researcher / Nurse / Fieldworker identify the diagnosis.

**IV. General QC instructions**

- 100% of each Study Researcher / Nurse / Fieldworker’s forms should be reviewed by the supervisor on at least a weekly basis, ideally, at the end of each working day. Supervisors should ensure the forms are complete (no missing fields), that the data appear to be correct, and that visits planned for the day were made.

- Corrections to the forms should be minimized (data should be correct the first time), but if needed, the Study Researcher / Nurse / Fieldworker should follow proper correction procedures. If form corrections are necessary:

* + 1. Cross through the incorrect information once,
    2. Write the correct information,
    3. Write the date the correction was made, and
    4. Write their initials.

- Make sure forms are kept confidential and protected (in a locked file drawer, for example) when they are returned from the field.

- Data center transmission of forms

* 1. Once forms have been completely filled out by the Study Researchers / Nurses / Fieldworkers and reviewed by the supervisor, they must be delivered within 48 hours to the local data center for data entry. Data entry of the forms should occur within one month of delivery to the data center (preferably sooner).

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
|  | Child ID | Write the child ID in the space provided at the upper left corner. |
| 01 | Study Researcher / Nurse / Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker’s unique ID number here. |
| 02 | Referral source | Enter here whether the child was referred for medical care by a Study Researcher/Nurse/Fieldworker (00) or if they sought care on their own (01). If no medical care was sought, but nutritional supplementation or medical treatment was given to the child, enter NA. |
| If the Study Researcher / Nurse / Fieldworker referred the child for medical care, fill in questions 3 and 5. | | |
| 03 | Referral date | Enter the date the child was referred for care. Format DD/MMM/YY |
| 04 | Today’s date | Enter today’s date. Format DD/MMM/YY |
| 05 | Visit healthcare provider | If the caregiver followed through with the referral and visited a health center based on the Study Researcher / Nurse / Fieldworker’s referral, enter 01 (Yes) and continue with the questions. If the caregiver did not seek out medical care, enter 00 (No). If the caregiver did not seek out medical care and the medical problem continues, the fieldworker should refer the child again for medical care (and fill out another Referral Form (REF)). |
| The following questions should be filled in for all reported interactions with health care providers (both referred by study staff and self-referred). Also, if the caregiver reports giving medication or nutritional supplementation to the child without consulting with the health care system, document this in questions 16-19. | | |
| 06 | Reason for seeking health care | Enter here the primary reason for seeking healthcare – if the primary reason for referral was diarrhea related illness, enter 01. If the primary reason for referral was ALRI related illness, enter 02. If the primary reason for referral was malaria, enter 03. If the primary reason for referral was poor growth, enter 05. If the primary reason for referral was anemia, enter 06. If the primary reason for referral was another reason (not 1, 2, 3, 5, or 6), enter 04 and write the reason in Q7.  If there is more than one reason for seeking care, enter the primary option in question 6 and write the other (secondary) option(s) in question 7 (other). |
| 07 | Other | If the reason for referral was not listed above (or if there was more than one reason), write the (secondary) reason here. |
| 08 | Date treatment was sought | Enter the date treatment was first sought. Format DD/MMM/YY |
| 09 | Source of health care | Enter here the source of healthcare – If the caregiver sought health care at a pharmacy, enter 01. If the caregiver sought health care at a formal health center or hospital, enter 02. If the caregiver sought care at another location, enter 03. |
| 10 | Diagnosis - dehydrating diarrhea | Enter here if the child was diagnosed with dehydrating diarrhea by a medical professional (doctor or nurse). Possible answers yes=01, no=00. |
| 11 | Diagnosis - ALRI | Enter here if the child was diagnosed with ALRI by a medical professional (doctor or nurse). Possible answers yes=01, no=00. |
| 12 | Diagnosis - Malaria | Enter here if the child was diagnosed with malaria by a medical professional (doctor or nurse). Possible answers yes=01, no=00. |
| 13 | Diagnosis – Other | Write the diagnosis here if the child was diagnosed with another condition by a medical professional (doctor or nurse). |
| 14 | Hospitalized? | Enter here whether the child was hospitalized as a result of this health center visit. Possible answers yes=01, no=00. |
| 15 | Hospitalized - # days | If the child was hospitalized, enter the number of days hospitalized here. |
| 16 | Nutritional supplementation | Enter 01 here if nutritional supplementation (e.g., pushti packet, vitamins, plumpynut, etc.) was given and write in supplement type in question 17. Enter 00 if no supplementation was given and go to question 18. |
| 17 | Supplement description | Write in type of nutritional supplementation that was given to the child. |
| 18 | Medication | Enter 01 here if medication (western or traditional) was given and write in medication type in question 19. Enter 00 if no medication was given. |
| 19 | Medication name | Write in type of medication that was given to the child. |

**MOD—Monthly Form**

**I. Purpose**

To document health status information and maintain contact with cohort children on a monthly basis from 37 to 60 months of age.

**II. Materials**

Monthly Form (MOD), MOD SOP, pen, clipboard

**III. Methods**

The Study Researcher / Nurse / Fieldworker will administer this form monthly when the child is 37 to 60 months of age. Ideally, the fieldworker will visit the home on the anniversary of the child’s date of birth (for a child born on May 17th, the fieldworker would visit on June 17th, July 17th, etc.) but it may not always be possible to visit on the exact day. At times when the exact anniversary date visit is not possible, the form can be filled out within two days before or after (for a child born on May 17th, the fieldworker can go between June 15th and June 19th for the 37 month visit, between July 15th and July 19th for the 38 month visit, etc.). It is very important that the form be completed within the given +/- two day window; however, if the visit is missed within that window, the Study Researcher / Nurse / Fieldworker may visit the household to collect the MOD information up to 7 days beyond the birthdate anniversary. If data collection occurs outside the +/- 2 day window, a PDF form must be completed.

**IV. General QC instructions**

* 100% of each Study Researcher / Nurse / Fieldworker’s forms should be reviewed by the supervisor on at least a weekly basis, ideally, at the end of each working day. Supervisors should ensure the forms are complete (no missing fields), that the data appear to be correct, and that visits planned for the day were made.
* Corrections to the forms should be minimized (data should be correct the first time), but if needed, the Study Researcher / Nurse / Fieldworker should follow proper correction procedures. If form corrections are necessary:

i. Cross through the incorrect information once,

ii. Write the correct information,

iii. Write the date the correction was made, and

iv. Write their initials.

* Make sure forms are kept confidential and protected (in a locked file drawer, for example) when they are returned from the field.
* Data center transmission of forms
  + Once forms have been completely filled out by the Study Researchers / Nurses / Fieldworkers and reviewed by the supervisor, they must be delivered within 48 hours to the local data center for data entry. Data entry of the forms should occur within one month of delivery to the data center (preferably sooner).

- Standard rounding techniques are recommended: Round down if last digit is <5 and round up if the last digit is >5).

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| 01 | Study Researcher / Nurse / Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker’s unique ID number here. |
| 02 | Today’s date | Format DD/MMM/YY |
|  | | |
| 03 | Has the child been sick at any time without hospitalization in the past 30 days  **If no skip to #07** | Yes = 01  No = 00 |
| 04 | If yes, what was the illness? (list all that apply) | Respiratory Tract Infection = 01  Diarrhea = 02  Fever = 03  Malaria = 04  Passed Worms = 05  Had Ear Discharge = 06  Other = 07 |
| 05 | If other, list illness |  |
| 06 | How many days during the last 30 days has the child been sick, without hospitalization? | Enter number of days (precede any number less than 10 with a 0; for instance, if the child has been sick for 2 days, enter 02) |
| 07 | Has the child been hospitalized at any time in the past 30 days?  **If no skip to #11** | Yes = 01  No = 00 |
| 08 | If yes, what was the diagnosis/illness? (list all that apply) | Respiratory Tract Infection = 01  Diarrhea = 02  Fever = 03  Malaria = 04  Passed Worms = 05  Had Ear Discharge = 06  Other = 07 |
| 09 | If other, write diagnosis |  |
| 10 | How many days during the last 30 was the child hospitalized? | Enter number of days (precede any number less than 10 with a 0; for instance, if the child has been hospitalized for 2 days, enter 02) |
| 11 | Has the child received any medications in the last 30 days?  **If no, skip to #13** | Yes = 01  No = 00 |
| 12 | If yes, list medications |  |
| 13 | Has the child received any antibiotics in the last 30 days?  **If no, form is finished** | Yes = 01  No = 00 |
| 14 | If yes, choose type of antibiotic | penicillin=01  cephalosporins=02  sulfonamides=03  macrolides=04  tetracyclines=05  fluoroquinolones=06  unknown=07  metronidazole=08  other=09  albendazole/mebendazole=10 |

**AVF – Anthropometry and Vaccine Form**

**I. Purpose**

To document height, weight, and vaccinations of children on a quarterly basis

**II. Materials**

Quarterly Anthropometry and Vaccine Form (AVF), AVF SOP, pen, clipboard

**III. Methods**

The Study Researcher / Nurse / Fieldworker trained in measuring anthropometry will administer this form quarterly when the child is 37 to 60 months of age. Ideally, the fieldworker will visit the home on the anniversary of the child’s date of birth (for a child born on May 17th, the fieldworker would visit on August 17th, November 17th, etc.) but it may not always be possible to visit on the exact day. At times when the exact anniversary date visit is not possible, the form can be filled out within two days before or after (for a child born on May 17th, the fieldworker can go between August 15th and August 19th for the 39 month visit, between November 15th and November 19th for the 42 month visit, etc.). It is very important that the form be completed within the given +/- two day window; however, if the visit is missed within that window, the Study Researcher / Nurse / Fieldworker may visit the household to collect the AVF information up to 7 days beyond the birthdate anniversary. If data collection occurs outside the +/- 2 day window, a PDF form must be completed. Ideally, the AVF should be completed on the same day the MOD is completed for that month.

The site should follow site-specific guidelines when poor growth is identified.

For vaccine information, the fieldworker should ask about vaccines administered since last visit – Ask to see vaccine records. If vaccine records are available, check to see if any vaccines have been given in the past three months. If vaccines have been given since the last quarterly visit, document the type of vaccine and date the vaccine was given here. If no vaccine records are available, ask the caregiver if the child has received any vaccines since the last quarterly visit, and if so, document the type and date of vaccination. If more than one dose was given of a particular vaccine since the last visit (because a quarterly visit was missed, for example), use the additional fields to include this information. If you only know the name of the vaccine but you do not know which diseases the vaccine protects against, write down the name of the vaccine on the Nursing Notes Form and discuss with your supervisor. For example, if the mother tells you the child received Pentaxim, you will enter the date for DPT, HiB, and IPV since pentaxim is a combination of all of those vaccines. If you don’t know what vaccines are included in a combination vaccine, ask your supervisor.

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| **#** | **Question** | **Guidance** | |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. | |
| 01 | Study Researcher / Nurse / Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker’s unique ID number here. | |
| 02 | Today’s date | Format DD/MMM/YY | |
| Vaccines administered in the past 3 months, write name and date | | | |
| 03 | Did the child receive any vaccines in the past 3 months?  **If yes, list below** | Yes = 01  No = 00 | |
| ***Instructions:*** *“***Source**”*refers to the source of the information regarding vaccinations. Vaccination information from vaccination cards or clinic records are STRONGLY preferred.*  *Options for* “**Source**”: ***0.*** *Unknown*, ***1.*** *From vaccination card,* ***2.*** *From clinic record,* ***3.*** *From verbal source (e.g. mother, guardian etc.),* ***4.*** *Other. There is space to enter 5 vaccines.* | | | |
| 04 | Name of vaccine | Date of vaccine (DD/MMM/YY) | Source |
| 05 | Name of vaccine | Date of vaccine (DD/MMM/YY) | Source |
| 06 | Name of vaccine | Date of vaccine (DD/MMM/YY) | Source |
| 07 | Name of vaccine | Date of vaccine (DD/MMM/YY) | Source |
| 08 | Name of vaccine | Date of vaccine (DD/MMM/YY) | Source |
| 09 | Weight (kg) | Enter here the value obtained by weighing the child’s weight in kilograms to two decimal places. | |
| 10 | Height (cm) | Enter here the value obtained by measuring the child’s height in centimeters to one decimal place. | |

**ANT – Anthropometry Check Form**

**I. Purpose**

Assess measurement error and identify training needs

**II. Summary**

Each month, a senior study nurse or field worker well-trained in anthropometry and who will be considered the gold standard should visit 5% of the households within 24 hours of the quarterly form data collection (AVF) in order to collect duplicate anthropometric measurements for quality control purposes. The 5% sample should be random and representative of the participants’ ages, locations, and all other variables.

**III. Methods**

Every quarter, the weight and height of 5% of the children will be collected using the ANT form and entered into the database. These anthropometric measurements will be used to assess measurement error and identify the need for retraining of staff. At the beginning of each quarter, the supervisor will calculate the number of participants in the study and then schedule quality control visits for a random sample of 5% of the participants to occur within 24 hours of regular administration of the AVF.

**IV. General QC Instructions**

QC of the anthropometry measurements will be done using the ANT form – In brief, 5% of the participants will have their anthropometric measurements taken a second time by a supervisor or someone with a high level of expertise in the collection of anthropometric data and these measurements will be compared (see ANT SOP for further details). Second measurements will be considered to be the gold standard.

* 100% of each Study Researcher / Nurse / Fieldworker’s forms should be reviewed by the supervisor on at least a weekly basis, ideally, at the end of each working day. Supervisors should ensure the forms are complete (no missing fields), that the data appear to be correct, and that visits planned for the day were made.
* Corrections to the forms should be minimized (data should be correct the first time), but if needed, the Study Researcher / Nurse / Fieldworker should follow proper correction procedures. If form corrections are necessary:

i. Cross through the incorrect information once,

ii. Write the correct information,

iii. Write the date the correction was made, and

iv. Write their initials.

* Make sure forms are kept confidential and protected (in a locked file drawer, for example) when they are returned from the field.
* Data center transmission of forms
  + Once forms have been completely filled out by the Study Researchers / Nurses / Fieldworkers and reviewed by the supervisor, they must be delivered within 48 hours to the local data center for data entry. Data entry of the forms should occur within one month of delivery to the data center (preferably sooner).
* Standard rounding techniques are recommended: Round down if last digit is <5 and round up if the last digit is >5).

**V. Additional notes**

The supervisor should review the ANT and AVF forms at the end of the day (or on a weekly basis) and discuss discrepancies found with the Study Researcher / Nurse / Fieldworker and provide additional training if necessary. Do not change the data that the Study Researcher / Nurse / Fieldworker collected!

**VI. Inconsistencies**

The original data collected by the Study Researcher / Nurse / Fieldworker will be entered into the database. There is no need to ‘correct’ the data based on the second data collector’s responses. This comparison is done simply to guarantee that the Study Researcher / Nurse / Fieldworker is making the visits and measuring the children, and for us to calculate measurement error and identify opportunities for additional training.

1. Minor inconsistencies

The supervisor should discuss the different measures with the Study Researcher / Nurse / Fieldworker. In many cases, the different measures may be due to the child’s state of mind, whether they were fidgeting or hungry or energetic and not wanting to be still for measurement. A small degree of variation is normal and does not need to cause concern.

1. Major inconsistencies

If there are large differences between the measurements (greater than 3 cm in height or 500 g in weight), the supervisor should ensure that a refresher training is completed by both Study Researcher / Nurse / Fieldworkers if both measurements were taken by Study Researcher / Nurse / Fieldworkers, or if the QC measurement was taken by the trainer (gold standard), the trainer should conduct the refresher training with the Study Researcher / Nurse / Fieldworker. If a Study Researcher / Nurse / Fieldworker consistently returns data that poorly measures anthropometry, and does not improve after additional training, that Study Researcher / Nurse / Fieldworker should be replaced.

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| --- | --- | --- |
| # | Question | Guidance |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| 01 | Study Researcher / Nurse / Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker’s unique ID number here. |
| 02 | Today’s date | Format DD/MMM/YY |
| 03 | Weight (kg) | Enter here the value obtained by weighing the child in kilograms to at least one decimal place. *Refer to SOP on anthropometry*. |
| 04 | Length (cm) in 0-24 months, height (cm) in 25-60 months | Enter here the value obtained by measuring the child’s length (0-24 months) or height (25-60 months) in centimeters to one decimal place. *Refer to SOP on anthropometry*. |
| 05 | Head circumference (cm) | Enter here the value obtained by measuring the child’s head circumference in centimeters to one decimal place. *Refer to SOP on anthropometry*. Enter NA for 37-60m extension period. |

**Dietary Assessments**

**NUT—Nutrition Form**

1. **Purpose**

There are methods for collecting dietary data during this time period: 1) an optional food frequency questionnaire (NUT) to be administered at 60 month follow up as part of the surveillance activity; 2) a series of three 24-hr dietary recalls (FRC & FRE) to be administered within -4 weeks/+ 2 weeks of the 60 month follow up and should take a maximum of four weeks for completion of all three recalls. FRC has two new questions added at the bottom compared to the FRQ form and the FRE form is exactly the same form as the previous FRS.

1. **Materials**

Nutrition Form (NUT), NUT SOP, pen, clipboard

1. **Methods: NUT form**

The monthly food frequency questionnaire is modeled after the MOA and MOC (used for same purpose for children 24 to 36 months of age), and is called the NUT. The field worker will administer this NUT form to the caretaker/mother monthly. Although there is a dietary assessment at 60 months, the NUT form should also be done at 60 months. This form is optional but is strongly recommended for sites to use. NUT and FRC forms can be collected on the same day.

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| **#** | **Question** | **Guidance** |
|  | Participant ID | Write the participant ID in the space provided at the upper left corner. |
| 01 | Study Researcher / Nurse / Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker’s unique ID number here. |
| 02 | Today’s date | Format DD/MMM/YY |
| The following questions should be asked about the diet yesterday (from yesterday sunrise until this morning sunrise). This first section asks only about the liquids the child has consumed. | | |
| 03 | Breast milk | Are you breastfeeding <CHILD>? Possible answers: 1=yes or 0=no. |
| 04 | Animal milk | Do you give <CHILD> animal milks, such as canned, powdered or fresh animal milk (cow, buffalo, goat, etc)? Possible answers: 1=yes or 0=no. |
| 05 | Plain water | Yesterday, during the day or last night, did <CHILD> have plain water? Possible answers: 1=yes or 0=no. |
| 06 | Tea, coffee, local examples | Yesterday, during the day or last night, did <CHILD> have tea or coffee or <local examples>? Possible answers: 1=yes or 0=no. |
| 07 | Fruit or vegetable juices? | Yesterday, during the day or last night, did <CHILD> have fruits or vegetable juice? Possible answers: 1=yes or 0=no |
| 08 | Carbonated beverages (Cola, Fanta, <local examples>)? | Yesterday, during the day or last night, did <CHILD> have any carbonated beverages (soda)? Possible answers: 1=yes or 0=no. |
| 09 | Thin soup or broth? | Yesterday, during the day or last night, did <CHILD> have any soup or broth: 1=yes or 0=no. |
| 10 | Rice, porridge, bread, noodles or other foods/drinks made from grains? | Possible answers: 1=yes or 0=no. |
| 11 | White potatoes, white yams, manioc, or other roots? | Possible answers: 1=yes or 0=no. |
| 12 | Carrots, squash, or sweet potatoes that are yellow or orange inside? | Possible answers: 1=yes or 0=no. |
| 13 | Any dark green leafy vegetables such as spinach? | Possible answers: 1=yes or 0=no. |
| 14 | Foods made with beans, lentils, peas, corn, ground nuts? | Possible answers: 1=yes or 0=no. |
| 15 | Ripe mangoes, papayas, or other sweet yellow, orange, or red fruit? | Possible answers: 1=yes or 0=no. |
| 16 | Any other fruits or vegetables such as banana, apple, oranges, tomatoes, avocado? | Possible answers: 1=yes or 0=no. |
| 17 | Liver, kidney, heart or other organ meats? | Possible answers: 1=yes or 0=no. |
| 18 | Any meat, such as chicken, beef, lamb, goat, duck (others)? | Possible answers: 1=yes or 0=no. |
| 19 | Eggs? | (includes eggs from chicken, ducks, etc) Possible answers: 1=yes or 0=no. |
| 20 | Fresh or dried fish or canned fish or shellfish? | Possible answers: 1=yes or 0=no. |
| 21 | Cheese, yogurt or other dairy products? | Possible answers: 1=yes or 0=no. |
| 22 | Any sugary foods such as pastries, cakes or biscuits? | Possible answers: 1=yes or 0=no. |
| 23 | Any salty foods such as chips, crisps, popcorn (local examples)? | Possible answers: 1=yes or 0=no. |
| 24 | Margarine, butter, or ghee? (as a spread) | Possible answers: 1=yes or 0=no. |
| 25 | Yesterday, counting meals and snacks, how many times did <CHILD> eat? | Enter here the number of times the child was fed yesterday (yesterday sunrise until this morning sunrise). Numbers include 00-99. |
| 26 | Was yesterday a typical day for the child? | Enter “01” if it usual intake that is typical of most days, “02” if the child was sick (vomiting, fever, diarrhea, etc), “03” if it was a holiday or other festivities or celebration and “04” if other reasons and enter the reason in question 27. |
| 27 | If 26 was ‘other’, fill in the reason here: |  |
| 28 | Child’s appetite | 4='very good' (likes to eat, interested in food, willing to try new foods); 3='good' (shows interest in food, eats well); 2='fair' (sometimes interested in food, doesn't like some foods); 1='poor' (not interested in new foods, likes a few foods,) |
| 29 | Vitamin A in the past 30 days | Possible answers: 1=yes if the child received vitamin A in the last month or 0=no. |
| 30 | Vitamin syrups | Enter “01” if ‘Regular' which is 2+ times/week. Possible answers: 1=yes or 0=no. |
| 31 | Iodized salt | Possible answers: 1=yes or 0=no. |
| 32 | Washed salt | Possible answers: 1=yes or 0=no. |
| 33 | Non-food items | Possible answers: 1=yes or 0=no. Some examples of non-food item include items like ash, chalk, clay, brick chips, paint chips, dirt, starch? |
| 34 | Yesterday, who took care of the child during all or part of the day? Enter as many as apply. | This question asks about childcare in the previous day and the questions can have multiple answers. Enter “01” if it was mother, “02” if it was sibling, “03” if it was other relatives in the household, “04” if it was relatives outside the household, “05” if it was unpaid non relative like friends, “06” if its paid child care, “07” if it was household staff, “08” if it was preschool and “09” if it was other. |
| 35 | Who provided information about the food your child ate yesterday? (either directly to the MAL-ED staff or the child’s mother) Enter as many as apply. | This question asks about all the caretakers who provided information on child’s feeding in the previous day and the questions can have multiple answers. Enter “01” if it was mother, “02” if it was sibiling, “03” if it was other relatives in the household, “04” if it was relatives outside the household, “05” if it was unpaid non relative like friends, “06” if its paid child care, “07” if it was household staff, “08” if it was preschool and “09” if it was other. |

**FRC.FRE SOP – Food Recall Forms**

**I. Purpose**

To document nutrient intake of the children at 60 months of age.

**II. Material**

Food recall forms (FRC and FRE), FRC.FRE SOP, pen, food models, clipboard

**III. Methods**

It is important to obtain quantitative estimates of the energy and nutrient intakes of the child around the time of the 60-month assessment. To capture the usual energy and nutrient intake, 3 independent (non-consecutive) 24 hour recalls should be collected. We will use the FRC and FRE forms for this purpose (they are similar to the FRQ and FRS forms used previously). All three recalls should be completed within 1 month (maximum of 30 days gap between the first and third recall). FRC has two new questions added at the bottom compared to the FRQ form and the FRE form is exactly the same form as the FRS. The Study Researcher / Nurse / Fieldworker trained in dietary assessment will obtain the 3 recalls around the time of the 60 month assessment. The following rules should be followed:

1. If the site feels that the 60 month cognitive assessment represents the final interaction with the child/family then the assessments should be done in the 2-3 weeks prior to the final assessment. If site agrees that the 60 month assessment is not the last contact per se, then at least one of the dietary assessments could follow the developmental assessment.

Timing of the surveys:

* They need to be taken on different days of the week, and only one should be a weekend (that word being culturally defined – so in Peru, that would be an interview on a Monday to capture Sunday’s intake, whereas in Pakistan it would be an interview on Sunday to capture Saturday’s intake).
* The recalls should be done on independent or non-consecutive days, meaning not Monday, Tuesday Wednesday, or Monday, Tuesday and Thursday, unless the Monday and Tuesday occur in different weeks.
* It IS okay to set up an appointment with the mother to help deal with the issue described below.
* It is advantageous to use a time period greater than 1 week to collect the data, so no need to try to group them together within the week prior to the developmental assessment.
* Sites will have to re-train on the technique and become familiar with the food codes now established through the analyses of dietary data from 9-24 months of age. Sites will have to identify whether changes in common utensils have occurred.
* It is OK to collect both NUT and FRC on the same day.

It may be true that the child goes to school or some other location and eats meals or snacks there, and the caregiver/mother doesn’t know what the child has eaten, and of course the child may consume snacks out and about (random child initiated eating events). There are 2 options: a) asking the child (semi-quantitatively) what they ate using utensils to semiquantitatively capture portions – this is the only way to get child initiated eating events; b) ask mother to find out the information about what the child usually eats at that location or ask the child to give the information (the fieldwork likely knows the activities of the child and the need to ask for this based on use of the MOD).

Other caretaker/child aiding in the recall:

Thus, at the beginning of the interview, the field worker should inquire in general about the child’s day to determine the extent to which it will be necessary to interview the child or have the mother get the information from someone else. As noted above, because the fieldworker can establish an appointment for the recall, she can orient the mother about obtaining information on her child’s intake from other care providers. This can be included in the notes portion on the FRC.

This special type of information (told by child/estimated by child or the mom knows what they ate but not in detail) can be included directly on the FRC with the following additional details:

1. Note under food/recipe description that it is reported by child/estimated by mother;

2. Use common code (e.g., 99999) for each of these items unless not necessary. For example, if the child says that they had a banana, then you could use the food code for banana;

3. Describe the amount served/left over in the appropriate boxes, and then if gram quantities are not known then use 999.9 as amount. Again, this is only for these special situations. For the banana example, the site may have already determined the gram amount for a banana, could record if the child ate all then the grams left over would be 0 (zero).

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
| 01 | Study Researcher/  Nutritionist/Fieldworker ID | Enter the Study Researcher / Nutritionist / Fieldworker’s unique ID number here. |
| 02 | Today’s Date | Format DD/MMM/YY |
| 03 | Child ID | Enter the Study Child’s unique ID number here. |
| 04 | Line number | This should be filled in as 1, 2, 3, etc., to enumerate each line of data. Sometimes, the interviewer will use extra space and thus, multiple lines may use the same number to reflect the continuation of information. Sometimes a food is a mix of two items and does not require a recipe form to be filled out, but rather the “food” is composed of two “ingredients” (so 2 line numbers for 1 food number) |
| 05 | Food number | This should be sequentially numbered for each food item, recipe or breast milk feed, etc. |
| 06 | Meal | Please identify each feeding episode/meal/snack separately. All foods/drinks that are offered together should get the same number. |
| 07 | Home | Where was the food consumed? Write “1” if it was consumed at Home and “0” if it was elsewhere. |
| 08 | Time | Record the time of the feed/offering of food or liquid. Our goal is to get relative times, we do not expect exact times here. |
| 09 | Recipe description | Name the recipe (this name should be the name given to this item on the recipe form) |
| 10 | Recipe code | This code will be given to the recipe after the interview |
| 11 | Food Item: description | Describe the food item sufficiently to allow identification of proper code |
| 12 | Food Item: Code | Enter the four digit code designated for this food. |
| 13 | (R/C):Raw/Cooked | Write “1” if the item was raw and “2” if it was cooked For example, cheese is considered “raw” but if its melted, then it’s considered “cooked” |
| 14 | Food served: Portion Size description | Describe what was served to the child; for example, the recipe code may refer to a vegetable soup, but the child only received the broth, or a potato that was then mashed up. |
| 15 | Food served: amount (in g) | Write down the amount in grams served if known, if not known, then column 13 should be sufficiently descriptive to allow a gram amount to be added after the interview is complete |
| 16 | Food leftover: Portion size description | Describe what amount of each food item was left over. For example, “¼ banana”, or they ate “almost all of it”, or they ate the peas out of the mashed potato only “. Do not leave this blank. If they ate it all, write “none left over” or “ate it all”. |
| 17 | Food left over amount (in g) | Write down the amount in grams left over if known, if not known, then column 15 should be sufficiently descriptive to allow a gram amount to be added after the interview is complete. For example, if the child were served ½ banana (under served question) and what was left was ¼ banana, then we would know they ate ¼ of a banana. Later in the office we would find that ½ banana is 20 g and then we would fill in that they were served 20 g of banana and there were 10 g left over and during analysis we would calculate that the child ate 10 g of banana. If the child “ate it all”, then record the number of grams left over as “0” (zero). Do not leave this blank. |
| 18 | Did the child have multiple caretaker(s) feeding the child yesterday? | Write “00” if the child only had mother or the main caretaker as the only provider of food intake in the previous day and “01” if child had multiple caretakers (besides mother or the main care taker). |
| 19 | Did the child aid in this questionnaire? | Write “00” if the child did not participate in the recall process and “01” if the child participated in this recall questionnaire – either (partially or the whole time) recalling the amount of food or snacks that was consumed randomly. |
| 20 | Comments | Please note if the child ate at the school/day care locations. Please also write down details if child aided in the 24 recall procedure in recalling the food consumed yesterday. Relevant details from interviewer or coder after the interview is complete such as special food offered to the child due celebration or other festivities. Ideally interviewer should conduct recall interview on day that is typical but if such instances, please note that in this section. |

**Cognitive Assessments**

**WPPSI—Wechsler Preschool and Primary Scale of Intelligence - III**

**I. Purpose**

The WPPSI-III is a clinical assessment tool that will be individually administered to each child at 5 years of age (±30 days). The WPPSI-III provides subtest and composite scores that represent intellectual functioning in verbal and cognitive domains and, when given in its entirety, a composite score that represents the child’s general intellectual ability. For the MAL-ED study, we will be administering eight subtests for the 4:0-7:3 age group (see section IV (Administering the WPPSI for MAL-ED) below for specifics).

**II. Testing Protocol**

The WPPSI-III requires a quiet space with good lighting. Therefore, this test will be administered at the central clinic location. A subset of the WPPSI-III administrations will be video recorded (as has been done for the Bayley and Raven’s from birth – 24 months) and the file will then be downloaded to a computer and stored until uploaded to DropBox for review by Dr. Murray-Kolb for QC purposes. Videos should be retained until feedback is received from Dr. Murray-Kolb and the site team has had a chance to review them. Once this is done, the files should be destroyed. The video camera should be set up so that it captures the entire testing area (the child, the table with the testing materials, and the tester). Ideally, we will have a good view of what the child is seeing and what answers the child is giving/pointing to (in other words, we will see the testing setup through the child’s eyes; this is needed so that we can check scoring). If there is a problem with the video equipment or there is another problem that precludes you from using the equipment (electrical outage, for instance), the test should still be administered. In other words, although we want to digitally record many of the testing sessions, we understand that issues will arise that preclude us from using the equipment and we do not want that to stop us from collecting the data. The Psychological Research Assistant (PRA) will conduct the test in a private room with only the child present. Establishing and maintaining rapport is key to eliciting the child’s cooperation and effort throughout administration of the test. For information about details of particular item administration, refer to the WPPSI-III manual.

The PRA will begin by introducing themself and explaining the test administration to the child. The official beginning of the testing session is reserved for establishing rapport between PRA and child. The PRAs will introduce the child to the WPPSI by saying the following:

**"I will be presenting game-like tasks to you. Some of the things I ask you to do will be easy, some might be hard, and some will be just right. I need you to try your best at all the tasks."**

**If a mother asks the PRA about the results of the assessment, or asks for a score or her child's IQ, the PRA should say:**

**"This test is being used for research purposes. We use it to get a picture of your child's abilities as compared with other children his/her age and not to get a particular score for your child.”**

**If a mother is particularly insistent, testers might continue:**

**"I am not qualified to interpret the results of any of the measures we use in this study. I would be happy to ask the SENIOR PSYCHOLOGICAL RESEARCH ASSOCIATE to talk more with you about the study."**

The pace at which the PRA presents items to the child is important in maintaining the child's interest and involvement. This pace should be adjusted to the individual child, but in general must be more rapid than an "ideal" interaction between an adult and child. The WPPSI-III is not an opportunity for children to explore materials, but an assessment of the child's developmental status. Items that are timed or that may require the child to use both hands should not be presented until the previous materials are removed. If a child seems to be getting frustrated or tired, it may be helpful to give him or her a small break before going on to the next task (please try to complete the subtest that is being worked on before giving the child a break).

Enthusiasm on the PRA's part is also important. It is especially crucial that the PRA's enthusiasm not vary with the child's success on particular tasks. Thus, the PRA must be just as likely to say "Good job" or "Nice work" when the child fails an item as when the child succeeds, as long as the child's attempts are directed toward the task. If the PRA is not able to do this, she should avoid using these phrases. In all cases, praise should be kept general and not include specific feedback. Focus should be kept on the child's effort or the attractiveness of the materials: "Great try" or "This is fun" or "You're working hard."

The WPPSI-III test materials must be organized systematically and thoroughly practiced so the testing runs smoothly. It is important to maintain standardized administration whenever possible, but deviations may be necessary especially when working with young children. Any deviations in the testing procedures should be noted on the Record Form and considered carefully when interpreting the test scores. The general administration procedures and specific subtest administration procedures in the Administration and Scoring Manual should be carefully reviewed prior to beginning the first assessment. All testing should be completed in one session if possible.

**III. Materials Needed**

WPPSI-III administration and scoring manual, Record form (for 4:0-7:3), Response booklet, WPPSI-III test kit, Stimulus Book 1, Stimulus Book 2, Block Design blocks (6 red, 4 white, 4 red & white blocks), # 2 pencil without eraser (bring extras), Scoring keys (for those sites administering Coding, Symbol Search), Clipboard, Stopwatch

**IV. Administering the WPPSI-III for MAL-ED**

As with the Bayley and the Raven’s tests, any individual who will administer the WPPSI-III in the MAL-ED study must first receive approval from Dr. Murray-Kolb’s team. This will occur after the testers have had enough time to practice with children until they feel that they have mastered administration. At this point, the tester will record their test administrations and the recordings will be sent to Dr. Murray-Kolb for evaluation. Recordings will be evaluated for accuracy of administration as well as scoring. Once the testers have mastered standard test administration (i.e., no administration or scoring mistakes) they will be “certified” as able to officially collect this data for the project.

In MAL-ED, we will administer the following subscales from the WPPSI-III **in the following order**:

* Block Design
* Information
* Matrix Reasoning
* Picture Concepts
* Word Reasoning
* Picture Completion
* Object Assembly
* Vocabulary (Nepal will use Comprehension in place of Vocabulary)

**V. Finishing the test:**

The entire test should be administered in one session. If the child appears to be getting tired or hungry, the PRA should take appropriate measures to remedy the situation. However, there are times when the PRA will try every possibility and will still not be able to get the child to finish the entire test in one session. In these instances, we would still rather get the data than lose it. Therefore, in the instances when all other options have been exhausted, the PRA will need to schedule a time for the child to complete the rest of the test. This testing session should be scheduled as close to the original testing session as possible. A protocol deviation form will then need to be completed.

**VI. Decisions agreed upon by MAL-ED Cognitive Subcommittee**

The following decisions were agreed upon by the MAL-ED subcommittee during the July 2014 training:

* Information:
  + #1: hamburger will be changed to banana across all sites
  + #2: bathtub, refrigerator, and stove are a problem; BG has replaced bathtub with a tap and bucket: BR says a shower should work; SA and TZ say a bucket should work; wording of question may need to change to “show me what you use for a bath” as opposed to “take a bath in”
* Matrix Reasoning:
  + #6: both PK and BG have changed; in PK bird to buffalo, worm to grass, squirrel to hen, and acorn to wheat; in BG bird to monkey, worm to banana, squirrel to goat, acorn to leaf; all other sites will adapt as needed
  + #7: smile will be removed (throughout); item to shade someone from sun/rain should be changed; BG and PK changed sunglasses to a hand-held fan; other sites indicate this might work for them (some indicate that the sunglasses are just fine); sites will adapt as needed
  + #10: BG changed to fish and fishhook and catapult and bird; therefore, they removed the butterfly; however, the butterfly distractor was included on purpose in this item; sites will adapt as needed
  + #15: change to local balls/sports things; original item gets at concept of going “through”; group decided that the concept that we will use is “targeting”; all sites to adapt such that the concept of targeting is present
* Vocabulary:
  + #3: 4 sites have changed the fork to a spoon; all other sites agree to do so also
  + #4: concerns about the depiction of the turtle; adaptations that have been used include a lizard, frog, crocodile, local turtle; all sites agree that they will use either a frog or a lizard
  + #5: original is a pumpkin; adaptations made have included eggplant, green pumpkin, carrots; suggestion of a tomato but concern that might look too much like an apple; each site will choose an appropriate vegetable to use for this item
  + #7: original is a telephone; all sites agree to change to cell/mobile phone; we’ll need to remember to change the scoring criteria based on a cell phone
* Picture Concepts:
  + #7: sink/pumpkin (use whatever you used to replace the pumpkin earlier); and calculator/tub; BG used hand pump for the sink and bucket of water for tub – we suggest BG changes the bucket of water to a pitcher of water; BR says they can use a sink and shower; NP a well and tap; TZ a pump and well; PK a faucet and pump; SA a well and tap; PE a tap and shower; IN a hand pump and tap
  + #15: original is hero which could be problematic for some sites; suggestions of using king or chief instead; group voted to keep this item as is
  + #12: skateboard/tricycle are problems; PK used a trolley; TZ says they could use a bicycle and a motorbike – suggestion that things with wheel that aren’t as obviously for transportation might be better; ideas are wheel cart/shopping cart/wheelbarrow; each site will adapt this item as needed
  + #13: the concept is that both have switches and they bring light; SA says they don’t really have switches at their site; TZ says picture 4 is ok but can replace other with small solar light or matchsticks; each site will adapt this item as needed
  + #15: original has ball/hockey sticks but they aren’t from the same sport; PK used a cricket ball and a bat but this needs to be change – they will use a bat and soccer ball; hamburger here will also be changed to a banana; rake may also need to be changed; each site will adapt this item as needed
  + #16: concept is that sun and candle both give off heat and light; no smiley on sun; change candle to local candle; change swimming pool to something local; each site will adapt as needed
  + #18: concept is that fire and sun are both sources of heat and light; replacement of pool needs to occur; hourglass may need to be replaced – suggest clock or watch
  + #19: key and electric can openers are both used to open things; sites will change to key and bottle opener or can (not electric) opener; BG indicates that they’ll have to think about this one
  + #25: sponge and boat are related to water; concept is of things related to water but in a different way; discussion of using a duck but doesn’t seem to work at all sites; lots of discussion and no agreement on finding something that will work at each site; suggestion to remove the item – 6 sites vote to eliminate the item so it will be eliminated
  + #27: this is supposed to be a very difficult item; the answer is dice with rolling pin with ball; concept is that they all roll in different ways; no rolling pin or dice in TZ or SA; BR tells their children to “play the dice” not “roll the dice”; BR suggests a bike in place of the dice (this will work for TZ and SA also); roller blades in the Spanish version and could be changed to a bicycle; IN needs to replace dice (bike will work); lots of discussion but couldn’t come up with something so will require more thinking
  + #28: basket, mailbox, and lunch bag go together; concept is 3 different types of containers that are used for different things; basket works everywhere but sites will change to be a representation of a local basket; final decision is that basket remains, mailbox becomes a water container/bottle/jug, and lunch bag becomes a shopping bag
* Word Reasoning:
  + #7: this is what you wear to keep your hands warm – glove doesn’t work at every site; IN changed to a sweater and “this is what you wear to keep your body warm” – it changed the difficulty and that’s OK because of re-ranking; can work in PK but they have to add “in the winter” to the question (the change that IN made will also work for them); sweater will work in TZ; children at the site in BR don’t need sweaters/gloves but may recognize from TV/movies; idea of socks was mentioned; sweater/gloves might work everywhere if we added the words “in the winter” to the end of the statement; sites voted for this last option
  + #9: this is a person who brings letters to people – mailman doesn’t work at the sites; will teach and teacher work at all sites? How about “this is a person who helps children learn in school”? Vote is to change to this final option where answer is teacher (try not to use the word “teach” in the statement)
  + #15: vacuum – this is supposed to be a more difficult item (this is something you plug in; this is something you plug in and it is used for cleaning); question: should emphasis be on plugging or cleaning? Several sites have changed this item to a broom – this has a long handle and it’s used for cleaning; some have said – this is something you plug in and it’s used to cool you down; PK has retained electric concept and used plug in and used to wash clothes (washing machine); this doesn’t work in several sites; electricity concept won’t work because of SA and TZ sites; back to broom – works in all sites! Decision to change the item to a broom
* Picture Completion:
  + #18: picture of lunch box with clasp missing; want to try to maintain the difficulty level; BG has used a local case with one latch and the other one missing – although it seems easier their data show that the difficulty has been retained; TZ suggests a hot pot which is a container with a latch to keep food warm; decision to all use any box with a latch that is appropriate for your site

#22: ice skating; NPK suggests 2 ladies wearing shoes with heels and one of the heels is missing; the group voted to use this option

**EFT – Executive Functioning Task**

**Executive Functioning Tasks** (instructions provided for the EF tasks in this SOP are adapted from: Obradovic, J., Yousafzai, A. K., Portilla, X. A., Rasheed, M. A., Memon, U., Siyal, S., Tirado-Strayer, N., & Keehn, B. (2014). School Readiness and Transition (STAR) Study: Executive Functions Battery Adaptation Manual. Early Childhood Cognitive Stimulation and Successful Transition to Preschool in a Disadvantaged Population in Rural Pakistan. Aga Khan University, Stanford University, & Harvard University. Grand Challenges Canada (#0061-03))

The Psychological Research Assistant (PRA) will administer 5 executive functioning (EF) tasks to the children at 60 months of age. These tasks are measures of various aspects of EF. The window period will be 30 days such that these tasks should be administered to the child at 60 months ± 30 days. The tasks should be done in a quiet, well-lit setting and should all be administered in one session. Therefore, these tasks will be administered at the central clinic location. The tasks to be done for the MAL-ED study (and the order in which they are to be administered) are: Day/Night Stroop, Dimensional Change Cart Sort, Forward Word Span, Go-No Go, and Forward Corsi Block Tapping. Information on each of these tasks is provided below. For a copy of the record forms and for more detail on the tasks, please see the above referenced document (Obradovic et al.) which has been posted to Central Desktop and distributed to all cognitive personnel.

**Testing Protocol**

A subset of the EF administrations will be video recorded (as has been done for the Bayley and Raven’s from birth – 24 months) and the file will then be downloaded to a computer and stored until uploaded to DropBox for review by Dr. Murray-Kolb for QC purposes. Videos should be retained until feedback is received from Dr. Murray-Kolb and the site team has had a chance to review them. Once this is done, the files should be destroyed. The video camera should be set up so that it captures the entire testing area (the child, the table with the testing materials, and the tester). Ideally, we will have a good view of what the child is seeing and what answers the child is giving/pointing to (in other words, we will see the testing setup through the child’s eyes; this is needed so that we can check scoring). If there is a problem with the video equipment or there is another problem that precludes you from using the equipment (electrical outage, for instance), the test should still be administered. In other words, although we want to digitally record many of the testing sessions, we understand that issues will arise that preclude us from using the equipment and we do not want that to stop us from collecting the data. The Psychological Research Assistant (PRA) will conduct the test in a private room with only the child present. Establishing and maintaining rapport is key to eliciting the child’s cooperation and effort throughout administration of the test.

The PRA will begin by introducing themself and explaining the test administration to the child. The official beginning of the testing session is reserved for establishing rapport between PRA and child. The PRAs will introduce the child to the EF tasks by saying the following:

**"I will be presenting game-like tasks to you. Some of the things I ask you to do will be easy, some might be hard, and some will be just right. I need you to try your best at all the tasks."**

The pace at which the PRA presents items to the child is important in maintaining the child's interest and involvement. The EF tasks are not an opportunity for children to explore materials, but an assessment of the child's developmental status. Items that are timed or that may require the child to use both hands should not be presented until the previous materials are removed. If a child seems to be getting frustrated or tired, it may be helpful to give him or her a small break before going on to the next task (please try to complete the task that is being worked on before giving the child a break).

Enthusiasm on the PRA's part is also important. It is especially crucial that the PRA's enthusiasm not vary with the child's success on particular tasks. Thus, the PRA must be just as likely to say "Good job" or "Nice work" when the child fails an item as when the child succeeds, as long as the child's attempts are directed toward the task. If the PRA is not able to do this, she should avoid using these phrases. In all cases, praise should be kept general and not include specific feedback. Focus should be kept on the child's effort or the attractiveness of the materials: "Great try" or "This is fun" or "You're working hard."

The EF test materials must be organized systematically and thoroughly practiced so the testing runs smoothly. It is important to maintain standardized administration whenever possible, but deviations may be necessary especially when working with young children. Any deviations in the testing procedures should be noted on the Record Form and considered carefully when interpreting the test scores.

**DNS – Day/Night Stroop**

**I. Purpose**

The Day/Night Stroop will be administered to the children as a measure of interference control

**II. Materials**

13 sun cards, 13 moon cards, DNS record form, DNS SOP, clipboard, pen

**III. Methods**

|  |  |  |
| --- | --- | --- |
| **STEP** | **MATERIAL** | **NUMBER** |
| Introduction | Sun cards | 1 |
|  | Moon cards | 1 |
| Practice Trials | Sun cards | 4 |
|  | Moon cards | 4 |
| Test Trials | Sun cards | 8 |
|  | Moon cards | 8 |
| Total | Sun cards | 13 |
|  | Moon cards | 13 |

**Set up**:

a. Take out cards with sun and moon on them and place in two piles in front of you. Keep cards face down until you present each card to child.

INTRODUCTION TRIALS 1-2

**“NOW WE’RE GOING TO PLAY A NEW GAME. I’M GOING TO ASK YOU SOME QUESTIONS FIRST.”**

|  |  |
| --- | --- |
| 1 | **“DO YOU KNOW WHEN THE SUN COMES OUT? IS IT IN THE DAY OR IN THE NIGHT?”** |
| 2 | **“DO YOU KNOW WHEN THE MOON AND STARS COME OUT? IS IT IN THE DAY OR IN THE NIGHT?”** |



*Record child’s response on scoring sheet*

* + - If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**
    - If INCORRECT say: **“THAT’S NOT QUITE RIGHT. THE [SUN/MOON] COMES OUT IN THE [DAY/NIGHT].”** *Do not repeat trial.*

INTRODUCTION TRIALS 3-4

**“OKAY, NOW WE ARE GOING TO PLAY A *SILLY* GAME WITH THESE CARDS.”**

b. Hold up a black moon card.

“IN THIS GAME, WHEN YOU SEE THIS CARD, I WANT YOU TO SAY ‘DAY’.”

3

**“SAY THE WORD ‘DAY’?”**



*Record child’s response on scoring sheet*

1. Remove card. Now hold up a white sun card.

“NOW, WHEN YOU SEE THIS CARD, I WANT YOU TO SAY ‘NIGHT’.”

4

**“SAY THE WORD ‘NIGHT?”**



*Record child’s response on scoring sheet*

* + - If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**
    - If INCORRECT say: **“THAT’S NOT QUITE RIGHT. REMEMBER, WHEN YOU SEE THIS** **CARD, YOU SHOULD SAY [DAY/NIGHT].”** *Do not repeat trial.*

1. Remove card.

PRACTICE TRIALS

1. Administer all practice trials by holding up one card at a time.

*Practice Trial 1*

|  |  |
| --- | --- |
|  | *Hold up the card. If child hesitates, say:*  **“WHAT DO YOU SAY FOR THIS ONE?”** |
| 1 | White sun card |



*Record child’s response on scoring sheet*

* + - If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**
    - If INCORRECT say: **“THAT’S NOT QUITE RIGHT. REMEMBER, WHEN YOU SEE THIS CARD, YOU SHOULD SAY NIGHT. [LET’S TRY AGAIN]”**

1. If response is incorrect, repeat practice trial one more time.
2. Proceed to the Practice Trial 2.

*Practice Trial 2*

|  |  |
| --- | --- |
|  | *Hold up the card. If child hesitates, say:*  **“WHAT DO YOU SAY FOR THIS ONE?”** |
| 2 | Black moon card |



*Record child’s response on scoring sheet*

* + - If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**
    - If INCORRECT say: **“THAT’S NOT QUITE RIGHT. REMEMBER, WHEN YOU SEE THIS CARD, YOU SHOULD SAY DAY. [LET’S TRY AGAIN]”**

1. If response is incorrect, repeat practice trial one more time.

**CHECKPOINT:**

If child got both Practice Trial 1 and 2 correct: If you repeated Practice Trial 1 and/or 2:

*Proceed to Test Trials Proceed to Practice Trials 3-4*

*Practice Trials 3-4*

“LET’S PRACTICE SOME MORE.”

1. Administer Practice Trials 3-4. Repeat practice trial one more time if response is incorrect.

|  |  |
| --- | --- |
|  | *Hold up the card. If child hesitates, say:*  **“WHAT DO YOU SAY FOR THIS ONE?”** |
| 3 | White sun card |
| 4 | Black moon card |



*Record child’s response on scoring sheet*

* + - If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**
    - If INCORRECT say: **“THAT’S NOT QUITE RIGHT. REMEMBER, WHEN YOU SEE THIS CARD, YOU SHOULD SAY [DAY/NIGHT. [LET’S TRY AGAIN]”**

1. Proceed to the Test Trials regardless of child’s performance on practice trials.

TEST TRIALS

**“OKAY, LET’S START THE GAME. READY?”**

1. Administer the test trials by holding up one card at a time.
2. *Do not use the words ‘day’ or ‘night’, and do not provide any feedback.*

|  |  |
| --- | --- |
|  | *Hold up the card. If child hesitates, say:*  **“WHAT DO YOU SAY FOR THIS ONE?”** |
| 1 | White sun card |
| 2 | Black moon card |
| 3 | Black moon card |
| 4 | White sun card |
| 5 | Black moon card |
| 6 | White sun card |
| 7 | White sun card |
| 8 | Black moon card |
| 9 | Black moon card |
| 10 | White sun card |
| 11 | Black moon card |
| 12 | White sun card |
| 13 | White sun card |
| 14 | Black moon card  *Record child’s response on scoring sheet* |
| 15 | White sun card |
| 16 | Black moon card |

6

**“GREAT JOB, YOU DID IT! LET’S MOVE ONTO THE NEXT GAME.”**

**DIMENSIONAL CHANGE CARD SORT (DCCS) GAME**

**I. Purpose**

The Dimensional Change Card Sort will be administered to the children as a measure of cognitive flexibility, assessing a child’s ability to switch between two different concepts and to think about multiple concepts simultaneously.

**II. Materials**

2 boxes, 1 red truck card, 1 blue star card, 8 blue truck cards, 9 red star cards, DCCS SOP, clipboard, pen

|  |  |  |
| --- | --- | --- |
| **STEP** | **MATERIAL** | **NUMBER** |
| Separated DCCS Introduction | Boxes | 2 |
|  | Red Truck | 1 |
|  | Blue Star | 1 |
|  | Red Star | 1 |
| Separated DCCS Practice Trials | Blue Truck | 2 |
|  | Red Star | 2 |
| Separated DCCS Test Trials | Blue Truck | 6 |
|  | Red Star | 6 |

**III. Methods**

**\*\*As a reminder: When sites trained on this task, they were taught to repeat the sort rule before each trial (in other words, remind the child of the sort rule). However, after piloting, it was determined that this made it too easy for the children and, therefore, we agreed to NOT repeat the sort rule before each trial.\*\***

1. Place sorting boxes on table. Box on the child’s left will have a blue card with a black star attached to the front. Box on the child’s right will have a red card with a black truck attached to the front.
2. NOTE: In all practice and test trials, cards should be sorted in box face down.

INTRODUCTION

**“HERE’S A BLUE STAR** (point to box on left) **AND HERE’S A RED TRUCK** (point to box on right)**.”**

“NOW, WE’RE GOING TO PLAY A CARD GAME. FIRST LET’S PLAY THE SHAPE GAME. IN THE SHAPE GAME, ALL THE STARS GO HERE (point to the box on the left) AND ALL THE TRUCKS GO THERE (point to the box on right).”

**“SEE, HERE’S A STAR** (hold up red star card)**. SO IT GOES HERE** (place it in box on left)**.”**

**“IF IT’S A STAR IT GOES HERE** (point to box on left)**, BUT IF IT’S A TRUCK IT GOES THERE** (point to box on right).**”**

SEPARATED PRACTICE TRIALS

*Practice Trial 1*

1. Hold up blue truck card as you say the prompt. Then hand the card to child to sort into the box.

1

**“NOW, HERE IS A TRUCK. WHERE DOES THIS ONE GO?”**



*Record child’s response on scoring sheet*

* + If CORRECT say: **“THAT’S RIGHT! YOU KNOW HOW TO PLAY THE SHAPE GAME.”**
  + If CORRECT but only points: **“CAN YOU HELP ME PUT THIS TRUCK IN THE BOX?”** (point to box on right)
* If INCORRECT say: **“THAT’S NOT QUITE RIGHT. THIS ONE’S A TRUCK, SO IT HAS TO** **GO OVER HERE IN THE SHAPE GAME** (point to box on right). **CAN YOU HELP ME PUT THIS TRUCK DOWN HERE? [LET’S TRY AGAIN]”**

1. If response is incorrect, repeat practice trial one more time.
2. Proceed to Practice Trial 2 regardless of child’s performance.

*Practice Trial 2*

1. Hold up red star card as you say the prompt. Then hand the card to child to sort into the box.

2

**“NOW, HERE IS A STAR. WHERE DOES THIS ONE GO?”**



*Record child’s response on scoring sheet*

* + If CORRECT say: **“THAT’S RIGHT! YOU KNOW HOW TO PLAY THE SHAPE GAME.”**
  + If CORRECT but only points: **“CAN YOU HELP ME PUT THIS STAR IN THE BOX?”** (point to box on left)
  + If INCORRECT say: **“THAT’S NOT QUITE RIGHT. THIS ONE’S A STAR, SO IT HAS TO** **GO OVER HERE IN THE SHAPE GAME** (point to box on left). **CAN YOU HELP ME PUT THIS STAR DOWN HERE? [LET’S TRY AGAIN]”**

1. If response is incorrect, repeat practice trial one more time.
2. Proceed to the Test Trials regardless of child’s performance on practice trials.

SEPARATED TEST TRIALS- SHAPE

**“NOW, IT’S YOUR TURN. SO REMEMBER, IF IT’S A STAR IT GOES HERE** (point to the box on left)**, BUT IF** **IT’S A TRUCK IT GOES THERE** (point to the box on right).**”**

1. For each trial, hold up the card, read the item, and then hand the card to child to sort into the box. *If child points to box as response, you may sort the card for child in that location.*
2. *Do not provide any feedback. You can say:* “**LET’S DO ANOTHER ONE”** or **“LET’S DO IT AGAIN”**

|  |  |
| --- | --- |
| 1 | (blue truck) **“HERE IS A TRUCK.”** |
| 2 | (red star) **“HERE IS A STAR.”** |
| 3 | (blue truck) **“HERE IS A TRUCK.”** |
| 4 | (blue truck) **“HERE IS A TRUCK.”** |
| 5 | (red star) **“HERE IS A STAR.”** |
| 6 | (red star) **“HERE IS A STAR.”** |



*Record child’s response on scoring sheet*

SEPARATED TEST TRIALS- COLOR

**“NOW WE’RE GOING TO PLAY A NEW GAME. WE’RE NOT GOING TO PLAY THE SHAPE GAME ANYMORE. WE’RE GOING TO PLAY THE COLOR GAME. IN THE COLOR GAME, ALL THE BLUE ONES GO** **HERE** (point to box on left)**, AND ALL OF THE RED ONES GO THERE** (point to box on right)**. REMEMBER, IF IT’S BLUE, PUT IT HERE** (point to box on left)**, BUT IF IT’S RED PUT IT THERE** (point to box on right)**. OKAY?”**

1. For each trial, hold up the card, read the item, and then hand the card to child to sort into the box. *If child points to box as response, you may sort the card for child in that location.*
2. *Do not provide any feedback. You can say:* “**LET’S DO ANOTHER ONE”** or **“LET’S DO IT AGAIN”**

|  |  |
| --- | --- |
| 1 | (red star) **“HERE IS A RED ONE.”** |
| 2 | (blue truck) **“HERE IS A BLUE ONE.”** |
| 3 | (blue truck) **“HERE IS A BLUE ONE.”** |
| 4 | (red star) **“HERE IS A RED ONE.”** |
| 5 | (red star) **“HERE IS A RED ONE.”** |
| 6 | (blue truck) **“HERE IS A BLUE ONE.”** |



*Record child’s response on scoring sheet*

**“GOOD JOB, YOU DID IT!”**

Remove all boxes and cards from table.

**Integrated DCCS**

**Materials**

2 boxes, 1 red boat card, 1 blue rabbit card, 9 blue boat cards, 8 red rabbit cards, DCCS SOP, clipboard, pen

|  |  |  |
| --- | --- | --- |
| **PROCEDURE STEP** | **MATERIAL** | **NUMBER** |
| Integrated DCCS Introduction | Boxes | 2 |
|  | Red Boat | 1 |
|  | Blue Rabbit | 1 |
|  | Blue Boat | 1 |
| Integrated DCCS Practice Trials | Blue Boat | 2 |
|  | Red Rabbit | 2 |
| Integrated DCCS Test Trials | Blue Boat | 6 |
|  | Red Rabbit | 6 |

**PROCEDURE:**

1. Place new sorting boxes on table. Box on the child’s left will have a white card with a blue rabbit attached to the front. Box on the child’s right will have a white card with a red boat attached to the front.
2. NOTE: In all practice and test trials, cards should be sorted in box face down.

INTRODUCTION

**“OKAY, LISTEN CLOSELY BECAUSE WE’RE GOING TO A PLAY A NEW GAME WITH SOME NEW CARDS.”**

**“HERE’S A BLUE RABBIT** (point to box on left) **AND HERE’S A RED BOAT** (point to box on right)**.”**

**“FIRST LET’S PLAY THE COLOR GAME. IN THE COLOR GAME, ALL THE BLUE ONES GO HERE** (point to box on left) **AND ALL THE RED ONES GO THERE** (point to box on right).”

**“SEE, HERE’S A BLUE ONE** (hold up blue boat)**. SO IT GOES HERE** (place it in box on left)**.”**

**“IF IT’S A BLUE ONE IT GOES HERE** (point to box on left)**, BUT IF IT’S A RED ONE IT GOES THERE** (point to box on right).**”**

INTEGRATED PRACTICE TRIALS

*Practice Trial 1*

1. Hold up red rabbit card as you say the prompt. Then hand the card to child to sort into the box.

1

**“NOW, HERE IS A RED ONE. WHERE DOES THIS ONE GO?”**



*Record child’s response on scoring sheet*

* If CORRECT say: **“THAT’S RIGHT! YOU KNOW HOW TO PLAY THE COLOR GAME.”**
* If CORRECT but only points: **“CAN YOU HELP ME PUT THIS RED ONE IN THE BOX?”** (point to box on right)
* If INCORRECT say: **“THAT’S NOT QUITE RIGHT. THIS ONE’S A RED ONE, SO IT HAS TO GO OVER HERE IN THE COLOR GAME** (point to box on right).

**CAN YOU HELP ME PUT THIS RED ONE DOWN HERE?”**

1. If response is incorrect, repeat practice trial one more time.
2. Proceed to Practice Trial 2 regardless of child’s performance.

*Practice Trial 2*

1. Hold up blue boat card as you say the prompt. Then hand the card to child to sort into the box.

2

**“NOW, HERE IS A BLUE ONE. WHERE DOES THIS ONE GO?”**



*Record child’s response on scoring sheet*

* If CORRECT say: **“THAT’S RIGHT! YOU KNOW HOW TO PLAY THE COLOR GAME.”**
* If CORRECT but only points: **“CAN YOU HELP ME PUT THIS BLUE ONE IN THE BOX?”**

(point to box on left)

* If INCORRECT say: **“THAT’S NOT QUITE RIGHT. THIS ONE’S A BLUE ONE, SO IT HAS TO GO OVER HERE IN THE COLOR GAME** (point to box on left).

**CAN YOU HELP ME PUT THIS BLUE ONE DOWN HERE?”**

1. If response is incorrect, repeat practice trial one more time.
2. Proceed to the Test Trials regardless of child’s performance on practice trials.

INTEGRATED TEST TRIALS- COLOR

**“NOW, IT’S YOUR TURN. SO REMEMBER, IF IT’S A BLUE ONE IT GOES HERE** (point to box on left)**, BUT IF IT’S A RED ONE IT GOES HERE** (pointing to box on right).**”**

1. For each trial, hold up the card, read the item, and then hand the card to child to sort into the box. *If child points to box as response, you may sort the card for child in that location.*
2. *Do not provide any feedback. You can say:* “**LET’S DO ANOTHER ONE”** or **“LET’S DO IT AGAIN”**

|  |  |
| --- | --- |
| 1 | (blue boat) **“HERE IS A BLUE ONE.”** |
| 2 | (red rabbit) **“HERE IS A RED ONE.”** |
| 3 | (blue boat) **“HERE IS A BLUE ONE.”** |
| 4 | (red rabbit) **“HERE IS A RED ONE.”** |
| 5 | (red rabbit) **“HERE IS A RED ONE.”** |
| 6 | (blue boat) **“HERE IS A BLUE ONE.”** |



*Record child’s response on scoring sheet*

INTEGRATED TEST TRIALS- SHAPE

**“NOW WE’RE GOING TO PLAY A NEW GAME. WE’RE NOT GOING TO PLAY THE COLOR GAME ANYMORE. WE’RE GOING TO PLAY THE SHAPE GAME. IN THE SHAPE GAME, ALL THE RABBITS GO HERE** (point to the box on left)**, AND ALL OF THE BOATS GO THERE** (point to the box on right)**. REMEMBER, IF IT’S A RABBIT, PUT IT HERE, BUT IF IT’S A BOAT PUT IT THERE. OKAY?”**

1. For each trial, hold up the card, read the item, and then hand the card to child to sort into the box. *If child points to box as response, you may sort the card for child in that location.*
2. Do not provide any feedback. You can say: “**LET’S DO ANOTHER ONE”** or **“LET’S DO IT AGAIN”**

|  |  |
| --- | --- |
| 1 | (red rabbit) **“HERE IS A RABBIT.”** |
| 2 | (blue boat) **“HERE IS A BOAT.”** |
| 3 | (blue boat) **“HERE IS A BOAT.”** |
| 4 | (red rabbit) **“HERE IS A RABBIT.”** |
| 5 | (blue boat) **“HERE IS A BOAT.”** |
| 6 | (red rabbit) **“HERE IS A RABBIT.”** |



*Record child’s response on scoring sheet*

**“GREAT JOB, YOU DID IT! LET’S GO ON TO THE NEXT GAME.”**

**FWS - WORD SPAN GAME**

**I. Purpose**

The Word Span Game will be administered to the children as a measure of the verbal, phonological loop system of working memory. Children will be asked to repeat a sequence of words in the exact same order.

**II. Materials**

Dog puppet, Word Span SOP, scoring sheet, clipboard, pen

**III. Methods**

INTRODUCTION

**“LET’S PLAY A GAME WHERE YOU COPY ME, OKAY?”**

Pull out puppet (dog)

**“HERE’S MY FRIEND.”** (dog says hi)

**“MY FRIEND IS GOING TO COPY ME FIRST, SO WHATEVER I SAY, HE SAYS TOO!”**

**“LIKE THIS. IF I SAY, ‘BEAR, HAT’—MY FRIEND SAYS:** (say in a different voice) **‘BEAR, HAT’.”**

FORWARD WORDSPAN (FWS) PRACTICE TRIALS

**“NOW YOU TRY.”** (put dog out of view)

**“I’LL SAY SOME WORDS AND THEN YOU SAY THOSE WORDS, JUST EXACTLY THE SAME WAY I DID.”**

**“FIRST, LET’S PRACTICE. LISTEN CLOSELY.”**

*Practice Trial 1*

1

**CAR SKY**



*Record child’s response on scoring sheet*

* If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**
* If INCORRECT say: **“THAT’S NOT QUITE RIGHT. I SAID ‘CAR, SKY’**

**SO TO SAY THEM JUST LIKE I DID, YOU WOULD SAY: ‘CAR, SKY’. [LET’S TRY AGAIN]”**

If response is incorrect, repeat practice trial one more time.

Proceed to Practice Trial 2 regardless of child’s performance.

*Practice Trial 2*

2

**DOG LEAF**



*Record child’s response on scoring sheet*

* If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**
* If INCORRECT say: **“THAT’S NOT QUITE RIGHT. I SAID ‘DOG, LEAF’**

**SO TO SAY THEM JUST LIKE I DID, YOU WOULD SAY: ‘DOG, LEAF’. [LET’S TRY AGAIN]”**

If response is incorrect, repeat practice trial one more time

CHECKPOINT:

If child got both Practice Trial 1 and 2 correct: *Proceed to Test Trials*

*Practice Trials 3-4*

If you repeated Practice Trial 1 and/or 2:

*Proceed to Practice Trials 3-4*

**“LET’S PRACTICE SOME MORE. LISTEN CLOSELY.”**

Administer Practice Trials 3-4. Repeat practice trial one more time if response is incorrect

|  |  |
| --- | --- |
| 3 | **CAR SKY** |
| 4 | **DOG LEAF** |



*Record child’s response on scoring sheet*

* If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**
* If INCORRECT say: **“THAT’S NOT QUITE RIGHT. I SAID [(CAR, SKY)** or **(DOG, LEAF)]**

**SO TO SAY THEM JUST LIKE I DID, YOU WOULD SAY: [(CAR, SKY) or (DOG, LEAF)]. [LET’S TRY AGAIN]”**

Proceed to the Test Trials regardless of child’s performance on practice trials.

FWS TEST TRIALS

**“GREAT! LET’S DO SOME MORE LIKE THAT.”**

**“REMEMBER, WHATEVER I SAY, YOU SAY IT THE SAME. ARE YOU READY? LISTEN CLOSELY.”**

Read words at an even pace from the scoring sheet, about 1 word per second.

Circle correct words, strike out incorrect words on scoring sheet.

Stop game if child fails 2 out of 3 items within a level.

|  |  |
| --- | --- |
| **LEVEL 2** | |
| 1 | **GAME CAP** |
| 2 | **SUN BOX** |
| 3 | **COIN BOOK** |
| **LEVEL 3** | |
| 4 | **TOY HILL MOON** |
| 5 | **KID FORK LEAF** |
| 6 | **LACE CLOUD BOAT** |
| **LEVEL 4** | |
| 7 | **BOY NIGHT BIRD SIGN** |
| 8 | **SHIRT JUICE WALL MOP** |
| 9 | **FARM LIGHT FOOT CRIB** |
| **LEVEL 5** | |
| 10 | **TENT GLASS DOG DRESS EYE** |
| 11 | **JET SAW BELL FLAG SAND** |
| 12 | **TREE MAN DRUM BUS FOX** |



*Record child’s response on scoring sheet*

**“GOOD JOB, YOU DID IT!.”**

**GNG - GO/NO GO (DONKEY) GAME**

**I. Purpose**

The Go/NoGo (Donkey) Game is an inhibitory control task which will be administered to the children as a measure of the child’s capacity for sustained attention and response control. The child must perform an action given certain stimuli and inhibit that action under a different set of stimuli.

**II. Materials**

Bell, Flip Book, Go/No Go (Donkey) SOP, scoring sheet, clipboard, pen

**III. Methods**

INTRODUCTION

Pull out the flip book and open it to the first Go/NoGo page with all the animals. Place between you and child, with the pictures facing the child.

**“HERE ARE SOME PICTURES OF ANIMALS.”**

**“HERE IS A CAT.”** (point to the cat)

**“HERE IS A DOG.”** (point to the dog)

**“HERE IS A TURTLE.”** (point to the turtle)

**“HERE IS A COW.”** (point to the cow)

**“HERE IS A FISH.”** (point to the fish)

**“HERE IS A BIRD.”** (point to the bird)

**“HERE IS A DONKEY.”** (point to the donkey)

Place the bell about 15 centimeters in front of the child.

**“IN THIS GAME, I WANT YOU TO PRESS THE BELL EVERY TIME YOU SEE AN ANIMAL BUT NOT WHEN IT IS A DONKEY.”**

**PRACTICE TRIALS**

|  |  |
| --- | --- |
| *For Practice Trials, you can provide the following response:*  *response:* | |
| Donkey Trial #8  If CORRECT, say: **“THAT’S RIGHT, GOOD JOB!”**  If INCORRECT, say: **“NO, YOU DON’T TOUCH THE BELL BECAUSE IT IS A DONKEY.”** | All Other Animal Trials #3 – 7  If CORRECT, say: **“THAT’S RIGHT, GOOD JOB!”**  If INCORRECT, say: **“YES, YOU TOUCH THE BELL BECAUSE IT IS NOT A DONKEY.”** |

Flip to next page with cat.

1

**“HERE IS A CAT. SHOULD YOU TOUCH THE BELL? YES, BECAUSE IT IS NOT A DONKEY.”**



*Record child’s response on scoring sheet*

Have child press the bell.

Flip to next page with donkey.

2

**“HERE IS A DONKEY. SHOULD YOU TOUCH THE BELL? NO, BECAUSE IT IS A DONKEY.”**



*Record child’s response on scoring sheet*

Flip to next page with dog.

3

**“HERE IS A DOG. SHOULD YOU TOUCH THE BELL?”**



*Record child’s response on scoring sheet*

Give child 2 seconds to respond. Correct as needed. Be sure child pressed the bell.

Flip to next page with turtle.

4

**“HERE IS A TURTLE. SHOULD YOU TOUCH THE BELL?”**



*Record child’s response on scoring sheet*

Give child 2 seconds to respond. Correct as needed. Be sure child pressed the bell.

Flip to next page with cow.

5

**“HERE IS A COW. SHOULD YOU TOUCH THE BELL?”**



*Record child’s response on scoring sheet*

Give child 2 seconds to respond. Correct as needed. Be sure child pressed the bell.

Flip to next page with fish.

6

**“HERE IS A FISH. SHOULD YOU TOUCH THE BELL?”**



*Record child’s response on scoring sheet*

Give child 2 seconds to respond. Correct as needed. Be sure child pressed the bell.

Flip to next page with bird.

7

**“HERE IS A BIRD. SHOULD YOU TOUCH THE BELL?”**



*Record child’s response on scoring sheet*

Give child 2 seconds to respond. Correct as needed. Be sure child pressed the bell.

Flip to next page with donkey.

8

**“HERE IS A DONKEY. SHOULD YOU TOUCH THE BELL?”**



*Record child’s response on scoring sheet*

Give child 2 seconds to respond. Correct as needed.

**CHECKPOINT:**

If child’s responses were all correct on Practice Trials:

*Proceed to Test Trials*

If child had any incorrect responses on

Practice Trials:

*Repeat all Practice Trials once from beginning*

Proceed to the Test Trials regardless of child’s performance on practice trials.

TEST TRIALS

**“NOW YOU ARE GOING TO PLAY ALL BY YOURSELF. I WILL SHOW YOU ANIMALS. PRESS THE BELL EVERY TIME YOU SEE AN ANIMAL BUT *NOT* WHEN IT IS A DONKEY. I’M NOT GOING TO SAY ANY MORE WORDS. PLAY ALL BY YOURSELF. READY? GO!”**

Flip page to Item 1 (turtle).

Allow the child 2 seconds to respond. Then turn the page even if the child has not responded. Proceed at this pace for all Test Trials.

Note on the scoring sheet whether or not the child touches the bell.

Always sco re the child’s first respo nse.

Do not identify the pictures of the animal to the child. If child fo rgets the rule, do n’t rem ind them of the rule.

**“GREAT JOB! LET’S MOVE ONTO THE NEXT GAME.”**

**CBT - CORSI BLOCK TAPPING GAME**

**I. Purpose**

The Corsi Block Tapping Game will be administered to the children as a measure of non-verbal, visuospatial work memory. Children will be asked to repeat block-tapping sequences of increasing length.

**II. Materials**

1 mat, 9 blocks affixed to mat, 1 wooden pencil for tapping, CBT SOP, clipboard, pen

|  |  |  |
| --- | --- | --- |
| **STEP** | **MATERIAL** | **NUMBER** |
| For complete test | Sheet | 1 |
|  | Blocks | 9 |
|  | Pencil for tapping | 1 |

**III. Methods**

PROCEDURE:

Pull out mat and nine blocks and place the apparatus between you and child. The numbers on the blocks should only face you. Pull out pencil with eraser tip.

NOTE: In all practice and test trials, you will tap the blocks in the sequence presented for each trial.

INTRODUCTION

**“NOW WE’RE GOING TO PLAY A NEW GAME.”**

**“THIS IS A SET OF BLOCKS. I’M GOING TO POINT TO SOME OF THE BLOCKS IN A CERTAIN ORDER WITH THE BACK OF THE PENCIL. AS SOON AS I FINISH POINTING, USE YOUR FINGER TO POINT TO THE SAME BLOCKS IN THE SAME ORDER. SO, JUST WATCH ME AND THEN DO WHAT I DID.”**

PRACTICE TRIALS

**“LET’S PRACTICE.”**

*Practice Trial 1*

1

**3 7**



*Record child’s response on scoring sheet*

If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**

If INCORRECT say: **“THAT’S NOT QUITE RIGHT. I TAPPED THESE BLOCKS** (tap blocks 3 and 7) **SO TO DO EXACTLY WHAT I DID, YOU WOULD ALSO TOUCH THOSE BLOCKS IN THAT ORDER. [LET’S TRY AGAIN]”**

If response is incorrect, repeat practice trial one more time.

Proceed to Practice Trial 2 regardless of child’s performance.

*Practice Trial 2*

2

**6 4**



*Record child’s response on scoring sheet*

If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**

If INCORRECT say: **“THAT’S NOT QUITE RIGHT. I TAPPED THESE BLOCKS** (tap blocks 6 and 4) **SO TO DO EXACTLY WHAT I DID, YOU WOULD ALSO TOUCH THOSE BLOCKS IN THAT ORDER. [LET’S TRY AGAIN]”**

If response is incorrect, repeat practice trial one more time.

**CHECKPOINT:**

If child got both Practice Trial 1 and 2 correct: If you repeated Practice Trial 1 and/or 2:

*Proceed to Test Trials Proceed to Practice Trials 3-4*

*Practice Trials 3-4*

**“LET’S PRACTICE SOME MORE.”**

Administer Practice Trials 3-4. Repeat practice trial one more time if response is incorrect.

|  |  |
| --- | --- |
| 3 | **3 7** |
| 4 | **6 4** |



*Record child’s response on*

*scoring sheet*

If CORRECT say: **“THAT’S RIGHT! GOOD JOB!”**

If INCORRECT say: **“THAT’S NOT QUITE RIGHT. I TAPPED THESE BLOCKS** (tap [blocks 3 and 7] *OR* [blocks 6 and 4]) **SO TO DO EXACTLY WHAT I DID, YOU WOULD ALSO TOUCH THOSE BLOCKS IN THAT ORDER. [LET’S TRY AGAIN]”**

Proceed to the Test Trials regardless of child’s performance on practice trials.

TEST TRIALS

**“GREAT! LET’S DO SOME MORE LIKE THAT.”**

**“REMEMBER, I’LL TOUCH SOME BLOCKS AND I WANT YOU TO TOUCH THEM EXACTLY IN THE SAME ORDER.”**

Tap blocks at an even pace in the order from the scoring sheet, about 1 block per second.

Circle correct blocks, strike out incorrect blocks on scoring sheet.

Stop game if child fails 2 out of 3 sequences within a level.



*Record child’s response on scoring sheet*

|  |  |
| --- | --- |
| **LEVEL 2** | |
| 1 | **5 7** |
| 2 | **2 1** |
| 3 | **8 2** |
| **LEVEL 3** | |
| 4 | **4 3 6** |
| 5 | **3 8 1** |
| 6 | **5 2 7** |
| **LEVEL 4** | |
| 7 | **3 2 4 9** |
| 8 | **1 7 6 5** |
| 9 | **3 5 7 1** |
| **LEVEL 5** | |
| 10 | **3 4 8 7 5** |
| 11 | **8 1 5 3 6** |
| 12 | **6 4 5 2 9** |

**“GOOD JOB! YOU DID IT!”**

**MAB—Movement Assessment Battery for Children-2**

* + - * 1. **Purpose and Background**

To document motor development of the children at 60 months of age.

The Movement Assessment Battery for Children-2 (MABC-2) will be administered by a Psychological Research Assistant (PRA) at age 5 (if resources and protocols allow). The collection window for each assessment is ± 30 days (eg 5 years ± 30 days). We have included this ‘window period’ to make scheduling a bit easier. If the mother or primary caregiver is unable to bring the child to be tested during this window period (for instance, if she is out of town), we will still collect this data as soon as she is available (for instance, when she returns). However, the date of test administration will be noted and therefore we will know that this data was collected at a slightly different time for that particular subject. If the date of the test administration is outside the ± 30 day window, a Protocol Deviation Form should be completed.

The MABC-2 for this age group encompasses 8 tasks under 3 headings. The first heading is *Manual Dexterity* and the tasks incorporated are Posting Coins, Threading Beads, and Drawing Trail. During Posting Coins, the child is to hold the box steady and place coins in box as quickly as possible when signaled. During the Threading Beads task, the child is to lace the beads on the thread, one at a time, as quickly as possible when signaled. In the third task, Drawing Trail, the child is to draw a single, continuous line following the trail from bicycle to house without leaving the boundaries. Note that there are a separate set of scoring guidelines for this task. The second heading encompasses *Aiming and Catching* which incorporates the tasks Catching Beanbag and Throwing Beanbag. In the first task, Catching Beanbag, the child must catch the beanbag thrown from the examiner with two hands. In the second task, Throwing Beanbag, the child must stand on one mat and underarm throw the beanbag to another target mat. The final heading is *Balance,* which incorporates the tasks of One Leg Balance, Walking on Heels, and Jumping on Mats. The first task is One Leg Balance where the child will stand on one leg on the mat. This task will be timed and recorded for each leg tested. In the second task, Walking Heels Raised, the child will begin at the start line with the toe of the leading foot and walk along the line with heels raised, not stepping off of the line. The final task is Jumping on Mats where the child will stand on the mat with feet together and make five consecutive jumps, landing each time with their feet together in a balanced position. For detailed directions on administration, see the MABC-2 manual.

The testing room for the MABC-2 requires a quiet space with good lighting (ideally the space be at least 6m x 4m with one smooth, blank wall). The floor should be relatively hard and smooth. A table and 2 chairs are needed- the height of the table/chairs should be appropriate for the child. An additional table to hold equipment is useful. There should be floor markings made prior to testing. If the mats are down, make sure they are taped down before testing. This test will be administered at the central clinic location.

It is recommended that the child wear clothing that allows them to move freely. Shoes are also recommended but testing can be done on a child who is barefoot. If the child

usually wears glasses, make sure they have them on during testing. The examiner should wear clothing that allows him/her to demonstrate the tasks.

A percentage of MABC-2 administrations will be video recorded and the file will then be downloaded to a computer and stored until sent to Dr. Murray-Kolb’s lab for QC purposes. These files should be transferred via Dropbox and an email sent to Dr. Murray-Kolb to let her know the names of the files that have been placed in Dropbox. The video camera should be set up so that it captures the entire testing area (the child, the table with the testing materials, and the tester). We need to be able to see what the child is doing/choices the child is making. If there is a problem with the video equipment or there is another problem that precludes you from using the equipment (electrical outage, for instance), the test should still be administered. In other words, although we want to capture a percentage of the testing sessions on video, we understand that issues will arise that preclude us from using the equipment and we do not want that to stop us from collecting the data. The PRA will conduct the test in a private room with only the child present. For information about details of particular item administration, refer to the MABC-2 manual.

The PRA will begin by introducing herself and establishing rapport with the child. The PRA should then explain the testing to the child by saying the following:

**"I will be presenting game-like tasks to you. Some of the things I give you might be easy, some might be hard, and some might be just right. Please try your best on all of the tasks.”**

The pace at which the PRA presents items to the child is important in maintaining the child's interest and involvement. This pace should be adjusted to the individual child. The MABC-2 is not an opportunity for children to explore materials, but an assessment of the child's developmental status. If a child seems to be getting frustrated, it may be helpful to give him or her a short break before going on to the next task. PRAs must remember that this takes time, however.

Enthusiasm on the PRA's part is also important. It is especially crucial that the PRA's enthusiasm not vary with the child's success on particular tasks. Thus, the PRA must be just as likely to say "Good job" or "Nice work" when the child fails an item as when the child succeeds, as long as the child's attempts are directed toward the task. If the PRA is not able to do this, she should avoid using these phrases. In all cases, praise should be kept general and not include specific feedback. Avoid saying, "You got all the coins in the box" or "You threw all the beanbags on the mat" or even "That's right." Instead, focus on the child's effort or the attractiveness of the materials: "Great try" or "This is fun" or "You're working hard."

1. **Materials:**

The materials required in the MABC-2 include:

* Record Form
* Timer
* Tape measure
* Colored tape (25 mm wide)
* Dark blue table- top mat
* AB1 Record Form with Drawing Trail Insert
* 1 Berol fine-tipped red pen
* 12 yellow coins
* Blue bank box (lid and base)
* 12 yellow beads
* Red lace
* Beanbag
* 6 floor mats
  + 3 solid yellow
  + 2 solid blue
  + 1 blue with orange target

All of the materials should be arranged prior to testing but should be outside of the child’s range of vision. Not each material is used for each individual task and should be arranged accordingly. The table-top mat that’s provided should always be used when child is performing the manipulative tasks (lining paper needs to be removed and placed with matte finish up and shiny side down). Examiners are cautioned to only use materials provided. All materials should be cleaned off after each child’s use, including the mats. It is preferable to use a diluted bleach solution for this purpose.

1. **Methods:**

There are 3 headings of 8 tasks completed in this assessment. The test items should be administered in the order that they appear in the manual. If the order needs to be changed, this must be noted.

*Manual Dexterity* is divided into 3 separate tasks. There should be a maximum of 2 formal trials allowed (when both hands are tested, that’s 2 formal trials for each hand). These tasks should follow the instructions in the MABC-2 manual, listed as follows:

* Posting Coins: pages 24-25
* Threading Beads: pages 26-27
* Drawing Trail: pages 28-29

*Aiming and Catching* is divided into 2 separate tasks. There should be a maximum of 10 formal trials allowed for each task. These tasks should follow the instructions in the MABC-2 manual, listed as follows:

* Catching Beanbag: pages 30-31
* Throwing Beanbag onto Mat: pages 32-33

*Balance* is divided into 3 separate tasks. There should be a maximum of 2 formal trials allowed (when both legs are tested, that’s 2 formal trials for each leg). These tasks should follow the instructions in the MABC-2 manual, listed as follows:

* One-Leg Balance: pages 34-35
* Walking Heels Raised: pages 36-37
* Jumping on Mats: pages 38-39

**Start point for test administration:** For purposes of the MAL-ED study, all children tested at the 5 year time point will be considered 5 years of age. Therefore, we will be using the tasks in Age Band 1 (3 – 6 years).

**Scoring:**

No assistance should be given for the formal trials that are being scored.

The record entry form should be filled out to monitor the child’s completion of the task as follows (see instruction manual for scoring of each task):

* Number of correctly executed attempts (if applicable) (0 is an acceptable answer)
* Preferred hand (if applicable)
* Number of seconds taken to complete each properly executed trial
* **F** for failure: if the child attempts the task but does not perform it properly
* **R** for refusal: can be used if the child refuses to attempt a task or trial
* **I**  for inappropriate: is used only when a child has a physical or sensory disability that renders the task inappropriate (unable to try the task)

*Scoring for Manual Dexterity 3- Drawing Trail (instructions can be found in Appendix A (pages 163-166) in the Examiner’s Manual)*

* Include all of the following scoring criteria listed above
* Failed trial (**F**) if the child:
  + Reverses direction while drawing
  + Turns paper more than 45 degrees
* If the child crosses the boundary with drawn line:
  + Running outside the boundary for less than 12 mm (0.5 in) counts as one error
  + When drawn line runs outside boundary for 12 mm or more, then an error is added for each 12 mm (for example, if outside line for 34mm, counts as 3 errors)
* If child starts or finishes more than 12 mm from the start/stop points, then one error is counted
* Arch: in the instructions, child is encouraged to continue the line “under and/or through the arch”; if child lifts pen and leaves a gap at the arch that is less than 12 mm, this is NOT counted as an error (if gap exceeds 12 mm then one error is counted)
* If child lifts pen but replaces it at exactly the same point, then this is NOT an error; if child lifts pen off paper and does not replace it at the same point, then an error is counted (regardless of how big the gap is)
* Overlapping or double lines
  + If there is an overlap of 1 mm or more when joined lines meet, an error is counted; an additional error is counted for each 12 mm that the overlap continues
* Crossing the boundary and leaving gaps
  + When children accidentally cross the boundary, they sometimes lift the pen to bring it back inside; if the join inside the boundary is neat and no gap is visible, then no error is counted for a gap but the child would get a score of 1 for crossing the boundary (provided it wasn’t 12 mm or more)
  + If, however, when the pen is replaced inside the line, a visible gap is left, an error is counted; this child would be given a score of 2, one for going outside the boundary (for less than 12 mm) and one for a discontinuous line
* Crossing the line and looping back
  + When the line crosses a boundary, the child may try to correct the error by looping back (thus reversing direction) to join the lines inside the boundary. If no gaps in the line are visible, then the child is only penalized for crossing the boundary (and you don’t measure the line in both directions for figuring out penalty score)
  + Failed trial
  + If child starts at correct point but picks up pen and starts drawing line in the opposite direction, the trial is failed
  + If the child turns the page more than 45 degrees, the trial is failed

*\*Note of Caution: if drawn line leaves a trace so big that it’s visible on both sides of the boundary line OR when the child pauses the pen leaves a blot so broad that it’s visible on both sides of the boundary line then the pen should be replaced and the trial begun again.*

**Finishing the test:**

The entire test should be administered in one session. If the child appears to be getting tired or hungry, the PRA should take appropriate measures to remedy the situation. However, there are times when the PRA will try every possibility and will still not be able to get the child to finish the entire test in one session. In these instances, we would still rather get the data than lose it. Therefore, in the instances when all other options have been exhausted, the PRA will need to schedule a time for the child to complete the rest of the test. This testing session should be scheduled as close to the original testing session as possible (within a couple of days, at most). A protocol deviation form will then need to be completed.

At the end of the record form, there are sections to record any non-motor factors that might affect movement (section IX) and any physical factors that might affect movement (X). For MAL-ED, these sections are optional. Sites may choose to complete the questions in these two sections if they wish.

**SDQ—Strengths and Difficulties Questionnaire**

**I. Purpose**

The SDQ will be administered to the mothers (or primary caregiver) as a brief behavioral screening of her 5 year old child. Twenty-five psychological attributes (some positive and some negative) are assessed.

**II. Materials**

SDQ Form, SDQ SOP, clipboard, pen

**III. Methods**

The SDQ will be administered by a Psychological Research Assistant (PRA) to the mother when the child is 5 years of age. The collection window for the assessment is ± 30 days (eg 5 years ± 30 days). We have included this ‘window period’ to make scheduling a bit easier. If the mother or primary caregiver is unable to be tested during this window period (for instance, if she is out of town), we will still collect this data as soon as she is able to be tested (for instance, when she returns). However, the date of test administration will be noted and therefore we will know that this data was collected at a slightly different time for that particular subject. If the date of the test administration is outside the ± 30 day window, a Protocol Deviation Form should be completed. This questionnaire can be used as a self-administered questionnaire or interviewer administered questionnaire. For purposes of consistency across the sites, we will employ the interviewer administered questionnaire technique.

There are 25 items on the SDQ, each scored 0, 1, or 2. A score of 0 indicates that this attribute is not true of the child, a score of 1 indicates that this attribute is somewhat true of the child, and a score of 2 indicates that this attribute is certainly true of the child. Therefore, the maximum score is 50.

Before administering this questionnaire, the PRA should be completely familiar with all items on the form. While administering the questionnaire, the PRA needs to remember to be objective and accepting (not approving/disapproving). The PRA will begin administration by introducing herself and explaining that she will describe several attributes (some positive and some negative) and the mother needs to think about the child when answering these questions. In order to ensure that each mother receives the same instructions, the following instructions will be read by the PRA to the mother:

**“The following questions are related to certain attributes that your child may or may not have. Please answer each question by telling me if this attribute is not true for your child, is somewhat true for your child, or is certainly true for your child over the past 6 months. Some of the attributes are positive attributes and some are negative. Please remember that children sometimes behave well and sometimes they do not. I am not here to judge your child but, rather, to collect accurate data on your child’s attributes. Therefore, please think about your child for each attribute that I list and answer truthfully. Please do try to answer every question that I ask you, even if you are not absolutely certain. Remember to answer each item on the basis of your child’s behavior over the past 6 months.”**

The PRA proceeds by asking every question on the questionnaire. After every other question, the PRA should remind the mother that the questions are asking about the child’s behaviors in the past 6 months. We want to make sure that the mother remembers that she is not reporting simply on the present. Once all of the questions have been asked and answered, the mother is free to discuss any of the questions with the PRA. This discussion time should not be used to change any of the previously given answers. Rather, this discussion time is used to allow subjects to feel that they are being listened to and serves as a way to discourage the mothers from insisting on discussion while the questionnaire is being administered.

**III. Scoring**

A total score as well as several subscale scores can be derived. The answers are summed in order to derive these scores. The following items are reverse coded:

#7, 11, 14, 21, and 25 (the database is programmed to do this; data entry personnel at each site need to enter the data exactly as recorded on the forms).

During the training that occurred for this questionnaire (July 2014), sites indicated that most of the items on this questionnaire were straightforward and should be understood by the mothers. However, there were a few instances where the sites felt that some guidance would be helpful. Please see guidance provided below for those particular questions:

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
|  | Participant ID | Write the participant ID in the space provided at the upper left corner. |
| Fieldworker ID | ### | Enter the Study Researcher / Nutritionist / Fieldworker’s unique ID number here. |
| Date | DD/MMM/YY | Format DD/MMM/YY |
| Identity of primary caregiver | Mother=01  Father=02  Grandmother=03  Grandfather=04  Aunt=05  Uncle=06  Other relative=07  Other nonrelative=08 | Write in code for identity of primary caregiver |

|  |  |  |  |
| --- | --- | --- | --- |
| **#** | **Question** | **Code** | **Guidance** |
| 1 | Considerate of other people’s feelings | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 2 | Restless, overactive, cannot stay still for long | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 3 | Often complains of headaches, stomach-aches or sickness | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 4 | Shares readily with other children, for example toys, treats and pencils | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 5 | Often loses temper | 00= Not True; 01=Somewhat True; 02=Certainly True | We are asking the mother if the child often becomes angry and expresses that anger by acting out |
| 6 | Rather solitary, prefers to play alone | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 7 | Generally well behaved, usually does what adults request | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 8 | Many worries or often seems worried | 00= Not True; 01=Somewhat True; 02=Certainly True | Some sites expressed concern that mothers will say that a 5 year old child is too young to worry; it might be helpful to ask the mother if the child worries about school or his/her friends, etc. |
| 9 | Helpful if someone is hurt, upset, or feeling ill | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 10 | Constantly fidgeting or squirming | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 11 | Has at least one good friend | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 12 | Often fights with other children or bullies them | 00= Not True; 01=Somewhat True; 02=Certainly True | Bullies could be defined as “threatens” or “demeans” or “intimidates” |
| 13 | Often unhappy, depressed or tearful | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 14 | Generally liked by other children | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 15 | Easily distracted, concentration wanders | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 16 | Nervous or clingy in new situations, easily loses confidence | 00= Not True; 01=Somewhat True; 02=Certainly True | Some translations said environment but there was concern that environment and situation aren’t interchangeable in each of the MAL-ED sites; be sure the mother understands that you’re asking about new situations |
| 17 | Kind to younger children | 00= Not True; 01=Somewhat True; 02=Certainly True | Here we are asking the mother to evaluate how the child acts with younger children; for example, does the child realize the younger child might need help with certain tasks and provides that (ie – is kind to younger children) or does the child ignore the younger child’s needs or perhaps even take advantage of the younger child (in these cases, the child would not be acting kindly) |
| 18 | Often lies or cheats | 00= Not True; 01=Somewhat True; 02=Certainly True | Presentation of this item to the mother will need to be done in a sensitive manner |
| 19 | Picked on or bullied by other children | 00= Not True; 01=Somewhat True; 02=Certainly True | Is the child threatened or demeaned by other children? |
| 20 | Often offers to help others (parents, teachers, other children) | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 21 | Thinks things out before acting | 00= Not True; 01=Somewhat True; 02=Certainly True | This item gets at the idea that the child thinks before s/he acts; in other words, they do not just spontaneously act without thinking through what the consequences might be |
| 22 | Steals from home, school or elsewhere | 00= Not True; 01=Somewhat True; 02=Certainly True | the child takes something from home, school, or elsewhere without asking |
| 23 | Gets along better with adults than with other children | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 24 | Many fears, easily scared | 00= Not True; 01=Somewhat True; 02=Certainly True |  |
| 25 | Good attention span, sees chores or homework through to the end | 00= Not True; 01=Somewhat True; 02=Certainly True | Does the child stay focused on his/her homework or household chores until they are finished (in other words, if the mother has to constantly remind the child to focus on what they are doing, then this child would not be considered as someone with a good attention span) |

**SDQ Versions**

There is also a Teacher Version of this questionnaire that can be administered. During the training, it was decided that many of the 5-year old children will not necessarily be in school at the MAL-ED sites and that, coupled with concerns about IRB issues, led to the decision to not include this version of the questionnaire as required for MAL-ED. However, specific sites may choose to administer the teacher version. There is also an impact supplement but it was decided that this would not work well in our MAL-ED settings. Therefore, this supplement will not be administered. The SDQ is freely available online with many official translations also available (these were distributed to the sites during the July 2014 training).

**HOM—HOME Inventory (Early Childhood)**

1. **Purpose**

The early childhood version of the HOME inventory will be administered by a Psychological Research Assistant (PRA) at 5 years ± 30 days. We have included this ‘window period’ to make scheduling a bit easier. If the mother or primary caregiver is unable to be tested during this window period (for instance, if she is out of town), we will still collect this data as soon as she is able to be tested (for instance, when she returns). However, the date of test administration will be noted and therefore we will know that this data was collected at a slightly different time for that particular subject. If the date of the test administration is outside the ± 30 day window, a Protocol Deviation Form should be completed. The HOME Inventory is designed to measure the quality of stimulation available to the child in the home.

1. **Materials**

Home Inventory Form (HOM), Home Inventory SOP, clipboard, pen.

1. **Methods**
2. The HOME Assessment should be scheduled at a time when the primary caregiver and child are in the home and the child is awake and not scheduled to be napping. When scheduling the appointment, the researcher will say the following to the mother: **“As we’ve done previously, we need to visit your home in order to see what the environment is like for your child. In order to do our assessment, we need to schedule a time with you when you and your child will be home and when your child will be awake and active. The assessment will typically take about 1 hour. During this time, we will be asking you some questions and observing your interaction with your child in your home. Therefore, we need to ensure that you and your child will be home during the entire time that we are completing our assessment. I’d like to go ahead and schedule a time for that assessment.”** The researcher will then schedule a time that is within 5 years ± 30days. Prior to the assessment, the researcher should remind the mother of the upcoming appointment.
3. Caregiver: The HOME is scheduled with the primary caregiver and child. In most situations, the primary caregiver will likely be the mother. However, there are times when the primary caregiver will be a grandmother, aunt, sibling, etc. If the primary caregiver identified is less than 12 years of age, the observation/interviewing will be done with the mother. Be sure to identify the primary caregiver whom you are interviewing/observing on the HOM record form.
4. At the beginning of the observation the PRA will tell the caregiver that s/he wants to watch the child’s daily activities for a while (about one hour) and that the caregiver will be asked a few questions. The PRA will also tell the caregiver that s/he would like to see where the child eats, sleeps, and plays. This will give the PRA access to the entire house and will enable the observation of things such as toys or books. The PRA will tell the caregiver to go about her work, but to keep the child at home (not let the child go to the neighbor’s house, etc.). The PRA will assure her that this is routine procedure for all children and families. If the family has a yard/courtyard-like area that is considered “part of the house”, the child and/or caregiver should feel free to spend time there during the assessment. They do not have to be indoors only, they just need to remain on the property so they can be observed.
5. The PRA should find a place to sit that is off to the side, but will give a good view of the house. The PRA should try to blend into the background.
6. The PRA should try not to stare at the child and family. Staring could make the family feel uncomfortable. It is OK to chat with the family a bit, especially at the beginning of the observation, to help them feel comfortable with your presence, then excuse yourself and say that you have some work to do. Your “work” is to watch the family, but be discrete. The PRA should be completely familiar with the instrument so that the initial chatting time can actually be used to gather information needed to complete the HOM. While you are working, do not smile or interact with the family – look down at your clipboard.
7. The PRA will record who s/he saw in the house during the observation (relationship to child and total number). Questions should be directed to the primary caregiver, if possible.
8. The PRA will complete each question of the Inventory by answering either no or yes. Some questions require observation only, some require interview, and some can be completed either through observation or interview (these options are marked on the answer form for each question). The interview questions can be integrated throughout the observation time but are generally asked at the end. This will give the PRA time to observe as much as possible and in the course of the observation/chatting with the primary caregiver, the PRA may get answers to many of the questions that are marked ‘interview’ and/or ‘either’. At the end, the PRA should check for those questions that still need answers and ask these to the primary caregiver. For questions that ask whether or not the caregiver does something (for example: “Parent answers child’s questions or requests verbally”, a positive score (score of 1) should be recorded if anyone present in the household (grandma, dad, aunt, older sibling, etc) does this activity. In other words, remember that we are interested in capturing the home environment from the CHILD’S point of view. If someone is answering the child, then this child is receiving that stimulation. The PRA does not have to observe the primary caregiver engaging in all of these activities in order to score positively (a score of 1), they just have to observe someone present engaging in these activities.
9. In order to ensure that each mother receives the same instructions, the following instructions will be read by the PRA to the mother:

**“I am here today to watch the child’s daily activities for a while (about one hour) and during this time I will ask you a few questions. In order to do my work today, I would like to see where the child eats, sleeps, and plays. While I do my work, please go about your regular work but be sure to keep CHILD’S NAME at home (don’t let the child go to the neighbor’s house, etc). This is routine procedure for all children and families and will allow me to get my work done in a timely fashion. I am going to sit off to the side so that I can do my work. I need you to go about your regularly scheduled activities while I’m here so that I can get an idea of what your child experiences every day. Please do not feel like you need to entertain me during this time. I will be doing my work. Do you have any questions before I begin?”**

1. Question guidance.

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| 01 | Study Researcher/  Nurse/Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker’s unique ID number here. |
| 02 | Today’s Date | Format DD/MMM/YY |
| 03 | Identity of primary caregiver (person being interviewed) | This question is to identify the primary caregiver, *ie.* the person to whom the questions are being addressed.  Enter the code for the caregiver: 01=mother, 02=father, 03=grandmother, 04=grandfather, 05=aunt, 06=uncle, 07=other relative, 08=other nonrelative. |
| 04 | # of people present during this observation period | Enter the number of people present in the household during any part of the interview, including the primary caregiver and study child. |
| 05 | Identity of those present in addition to the child and primary caregiver | Identify ALL of the people present *in addition* to the caregiver. Include anyone present for any part of the interview. Do not include the primary caregiver here. Use the following codes: 01=mother, 02=father, 03=brother, 04=sister, 05=grandmother, 06=grandfather, 07=aunt, 08=uncle, 09=cousin, 10=other relative, 11=other nonrelative. |
| The possible answers for all of the following questions are: Yes=01, No=00 (with the exception of question 13 where the number of books in the home needs to be recorded). | | |
| 06 | Child has toys which help teach colors, sizes, OR shapes. | This does not have to refer to one toy that teaches all these things. However, if the parent has bought a single toy that does, credit should be given. Examples of individual toys that merit credit on this item are shapes sorting cubes, press-outs, mailbox, pegboards and many commercially available items. A toy that may not be specifically designed to teach colors, sizes, or shapes but that the parent uses in such way may also get credit. For example, if the child has a blue ball and the parent says, “what color is this ball?” or “look, this ball is blue” that would be an indication that the parent is using one of the child’s toys to teach him/her colors. Or, using the same blue ball, the parent might say, “what shape is this ball?” or “this ball is round” and that would indicate that the parent was using the ball to teach shapes. Just the presence of the blue ball is not sufficient to give credit for teaching color or shape. The observer must see the parent use that toy in such a way as to teach colors, shapes, or sizes. |
| 07 | Child has three or more puzzles or toys that the child must “put together”. | The puzzles/toy must be appropriate to the child’s age-challenging, yet accomplishable. All the pieces must be with the puzzle. Many times a parent will say, “Oh, yes, he has lots of puzzles, but I don’t know where the pieces are.” Thus it is a good idea to inquire about whether all puzzle parts are still present. |
| 08 | Child has a record, tape, radio or CD player and at least five children’s records, tapes, songs regularly listened to or CD’s. | The machine may be that of the parents or the whole family as long as the child has his own records, tapes, or CD’s and is permitted to use the machine. |
| 09 | Child has toys or games permitting free expression. | Examples of toys allowing free expression would be clay, finger paints, play dough, crayons, and paint and large pieces of paper. The child must have at least two different types of toys or materials that encourage self-expression. |
| 10 | Child has toys or games requiring fine movements. | The key to this item is whether the toy or material encourages fine as opposed to gross motor development. Credit should be given for any materials which require a certain amount of control in their use. Examples are small building materials, train sets requiring assembly, dolls with clothes that can be put on and taken off, coloring paper, crayons, scissors and paper, and stringing beads. Child must have at least **two** such items. |
| 11 | Child has toys or games which help teach numbers. | This could include puzzles with numbers, blocks, books, games, computer games, dominoes, and playing cards. Having books alone is not enough. Books plus one of the other types of toys could receive credit. |
| 12 | Child has at least one children’s book. | These must be children’s books and must be in readable condition. They need not be the sole property of the child but may be shared with siblings or have been handed down by older children. The books must be developmentally appropriate. If the child is visually impaired, stories on records, tapes, or CD’s are acceptable. |
| 13 | How many books are visible in the apartment or home? | The word “visible” was used in this item to make it an observation rather than an interview item if possible. However, being able to observe without going through the entire home is usually difficult. In such instances do not hesitate to ask about the reading habits of the family. In other words, if books aren’t visible, you may ask the parent to show you where they keep books. The intent of the item is to find out something about whether the child is growing up in a family that reads and values having books around. There might be a set of encyclopedias on the shelf which appears to be unopened. Nonetheless, presence of the books would indicate that the family values their possession and credit would thus be given. Certain high quality magazines, such as *National Geographic*, which are often treated as books and saved, can also be counted. The books need not be the private property of the immediate family so long as they are present in the home. |
| 14 | Family buys and reads a newspaper regularly or one of the parents goes to a local coffee shop to read a newspaper regularly. | This item is also designed to get at the reading habits of the family. The question should not be scored plus unless the paper is read regularly. It does not have to be read in its entirety, but the news should be sampled fairly completely (more than comics and TV section). It is acceptable if only one parent reads the paper. |
| 15 | Family subscribes to or regularly purchases at least one magazine. | When discussing books it is usually easy to ask whether the parent ever finds time to read magazines. Any magazines the family might subscribe to or regularly purchase are acceptable, including children’s magazines. |
| 16 | Child is encouraged to learn shapes. | This item can be easily approached in the discussion of learning materials. What is sought here is whether the parent makes a conscious effort to help the child recognize different shapes and know what they are called; one way to do this is by the purchase of toys that are clearly designed to teach shapes. But it will also be manifested by behavior on the parent’s part. She might mention that “A ball is round,” or “That block is square” when playing with the child. With an older child who uses paper and pencil, the mother might take the time to draw and label different shapes for the child. To help get the necessary information the Visitor might ask, “*Does \_\_\_\_\_ know things like “the ball is round” – “the box is square” and other shapes? How did s/he learn that?* |
| 17 | Child has toys that help teach the names of animals (or the family has several animals). | Examples include toy animals, books about animals, circus games, animal puzzles, or live animals. To give credit for this item, the child should have at least **two** of these toys or animals. |
| 18 | Child is encouraged to learn the alphabet. | For this item, any attempt to introduce the child to the letters is acceptable. Teaching her to print letters or her name, talking about and pointing out letters in books or magazines, and playing with a chalk board are common forms of teaching the letters. Singing songs about the alphabet or a computer game/TV show that teaches the alphabet are also acceptable. |
| 19 | Parent teaches child simple verbal manners-(please, thank you, I’m sorry). | Mandates (commands) do not count as teaching. Most attempts to teach manners are acceptable. However, if all the parent has done is to command- e.g., “Say yes ma’am” or “Say no ma’am”—thereby establishing the parent’s superior status, that is not sufficient. Instruction in several different forms of courtesy or politeness is required—even if the child hasn’t learned it yet. |
| 20 | Parent encourages child to talk and takes time to listen. | This is designed to find out whether the parent takes an active interest in the child’s experiences and activities. Does she actively inquire about what he did when he was across the street at a friend’s birthday party or what he did at school in the morning? In order for this to receive credit the parent must make an active effort to have the child relate experiences. |
| 21 | Child is permitted choice in breakfast or lunch menu. | “Permitted” is the key words in this item. The child does not have to order special menus in order for credit to be given and the parent does not have to give in to what the child chooses. We are looking for instances where the child’s wishes receive any consideration. |
| 22 | Parent uses correct grammar and pronunciation. | To receive credit the parent must be able to communicate with the Visitor. Pronunciation with enough precision that the mother can be understood is more important than precise grammar. Dialectical differences should not be a consideration. The issue is the ability to communicate clearly and with precision. |
| 23 | Parent’s voice conveys positive feelings about child. | Is the parent pleased with the child? Does she enjoy her and talk about her in a pleasant, joyful manner rather than talk in a flat tone which communicates, “She’s here, so I’ll put up with her.” |
| 24 | Building appears safe and free of hazards. | There is no plaster coming down from ceiling, a stairway with boards missing, rats running around, etc (in other words, things aren’t falling apart in such a way that they could cause harm to someone). The Visitor should use her own good judgment in scoring. Some of the most common concerns for this item are: open gas fires in small homes; exposed insect or animal traps; frayed electrical cords; and the presence of bleach, cleaning fluids, poisons, or sharp kitchen utensils within easy reach of a small child. Overcrowding or clutter in the home does not count as a hazard unless it is to such an extent that it could injure the child. |
| 25 | Outside play environment appears safe. | (No available outside play area requires an automatic zero score.) Once again the Visitor should use good judgment in the scoring. Examples of typical hazards are: broken glass or metal lying around, junk cars abandoned in the yard or along the side of the street, open ditches or a house so close to a street that a child could not safely play in the yard, and boards with nails sticking up out of them. The outside play area need not be adjacent to the home, but must be within a very short walking distance (e.g., one block). |
| 26 | The interior of the home or apartment is not dark or perceptually monotonous. | On this item the interviewer can take into account the lack of lighting, drawn drapes, lack of pictures or plants, or a seeming lack of effort to liven up the home and make it attractive. Perceptual monotony could also include a home where there were very few furnishings or they were all in the same general color. |
| 27 | Neighborhood is aesthetically pleasing. | Items such as trees and grass in the neighborhood would be considered aesthetically pleasing. Neighborhoods with lots of junk cars and garbage or other debris would be considered a negative and scored a zero. Unsightly and/or dilapidated housing will be scored a zero. |
| 28 | House has at least 100 square feet of living space per person. | In making a rough calculation for this item, we use as a general rule of thumb a 3 x 3 meter or a 2.7 x 3.7 meter room as being about the right amount of space for **one** person. If the courtyard is used by the family as living space, please be sure to include this space when evaluating this item. |
| 29 | Rooms are not overcrowded with furniture. | Is the furniture arranged in a manner so that all of the exits are free and easily accessible? This can be crucial in the event of a fire or other type of natural disaster. Does the living area allow for freedom of movement and room for the children to play, unless another specified area is designed as a play area? |
| 30 | House is reasonably clean and minimally cluttered. | The Visitor will have to use her own good judgment in scoring this item. Do not score a one if rooms are clearly unsanitary (e.g., presence of many bugs, food on floor, piles of dirty dishes in sink, noticeable odor of urine or garbage). Other minuses include piles of newspapers or magazines or clothing that has not been put away. |
| 31 | Parent holds child close 10-15 minutes per day. | This does not have to be done all at one time. A couple of minutes several times a day will receive credit. The father may find time to hold the child and talk when he comes home from work or they may sit down and watch TV or look at a book together. If the child lives with grandparents, their attention also counts. |
| 32 | Parent converses with child at least twice during visit. | This item involves parental conversation, not just vocalization which can be any sounds or words exchanged with the child. Also, scolding and accusatory comments are not counted. The parent must make an effort to converse with the child and ask questions, talk about things, or engage in verbal interchanges other than scolding or degrading comments. |
| 33 | Parent answers child’s questions or request verbally. | In order to receive credit for this item the parent must make an effort to answer some questions asked by the child. If unable to answer it at the moment, the parent may tell the child she doesn’t know but that they will look up the answer later. Responses such as “Sh-h! I’m talking,” “Mother’s busy, go away,” “Don’t bother me now” do not receive credit. It is rare for a child not to ask a question, request permission, etc., during a one-hour visit. However, if the child does not ask a question during the visit, this item should be scored positively (score of 1). |
| 34 | Parent usually responds verbally to child’s speech. | The key here is that the parent recognizes and acknowledges the child’s vocalization and does not ignore them. For a score of 1, the response may be only a word or series of words or sounds such as, “Uh huh,” “Um” or “Sure.” If the child does not vocalize in any way during the interview, thereby giving no opportunity for response, the score would be a 0. |
| 35 | Parent praises child’s qualities twice during a visit. | A key element here is the spontaneity of the praise. Since most mothers enjoy talking about and are proud of their children, this is not too hard to observe. Frequently a mother will speak with pride about how well her child throws a ball or runs and will brag on how well he dresses himself or can feed himself. Any of these would earn credit but you must be sure that the same person praises the child **twice** during the visit in order to score a 1 (it cannot be two different people who each praise once). |
| 36 | Parent caresses, kisses, or cuddles child during visit. | This need not be a wild burst of showy affection. Simple signs of concern such as a mother gently tucking the child’s shirt in, holding him on her lap, holding a hand, or a gentle pat on the shoulder would all receive a plus. We are looking for signs of physical affection and there must be at least one such incident in order to score a 1. |
| 37 | Parent helps child demonstrate some achievement during visit. | Note whether the parent consciously gets the child to sing a song, count, show how a toy works, or do anything that allows her to do something to impress the Visitor. All that is important is the parent’s attempt to get the child to show off, not whether the child actually does it. |
| 38 | Child is encouraged to learn colors. | Any attempt by the parent to teach colors will receive credit. Common times are when the child is being dressed, when playing with toys, or watching cars go by. The key here is whether the parent is actively involved in helping the child learn about colors. |
| 39 | Child is encouraged to learn patterned speech. | This includes nursery rhymes, prayers, songs, TV commercials, etc. Frequently this item can be scored by bringing the child into the interview and asking him if he knows any songs or nursery rhymes. The mother will usually beam with pride and encourage the child to sing or recite a poem. Many families say a blessing before meals, and the children are encouraged to join in. Also many children learn and are able to repeat the popular TV commercials. Be sure to inquire where the child learned these, as they may have been learned at church or in child care. If this is the case, be sure to continue to probe to find out whether any family member attempts to teach the child any of these things. We are looking for instances where the child is encouraged to learn patterned speech in his/her home (not by someone outside of the home). |
| 40 | Child is encouraged to learn spatial relationships (up-down, under-over, big-little, etc.). | Most parents do this without really being aware of it. A child is told to pick “up” his toys and put them “in” the box. However, credit should be given only if there is evidence of deliberate and planned clarification of the meaning of these confusing prepositions. In other words, it’s obvious that the parent is trying to teach the child the meaning of these words. |
| 41 | Child is encouraged to learn numbers. | Any attempt at teaching the child numbers is given a score of 1. Counting the child’s toes or fingers aloud, asking the child, “How old are you?” and teaching her to show by holding up a certain number of fingers are examples of such activities. |
| 42 | Child is encourages to learn to read a few words. | The “teaching” referred to in the item does not mean formal instruction, and it certainly doesn’t mean “pushing” the child to read. It merely refers to whether parents take advantage of daily opportunities (grocery shopping, story reading session, etc.) to call to the child’s attention the association between printed letters and sounds. One would not expect many homes to receive credit on this item at the lower end of the early childhood years. Examples of actions that would receive credit would be reading alphabet books, giving the child paper and pencil and letting her copy letters, responding to the child’s question, “What’s that word?” when reading stories, etc. Remember that we are only giving credit if the effort to encourage learning and reading a few words is done in the home (teaching at school does not count). |
| 43 | Some delay of food gratification is expected. | This is one of those items we would just as soon had not met the criteria that mandated its inclusion in the scale. We knew what we meant by it, but it is somewhat difficult to translate what we meant into the words we used to convey that meaning. One indication of increasing maturity is a lengthening of one’s ability to delay gratification. From this we reasoned that one indication of a home environment that could foster maturity is the expectation, somehow communicated to the young child, that he or she should be able to delay gratification. One operational indication of this expectation would be a refusal to tolerate whining during the last 30 minutes of meal preparation (sending child out of room, etc.). It is possible that this item can be scored during a broad discussion of food and eating habits. However, if not, the Visitor should inquire directly as to whether the child snacks any time he is hungry or whether house rules call for waiting until meal time. |
| 44 | TV is used judiciously. | The key word here is “judiciously.” If the family does not have a television the score would be 0. Likewise, almost any attempt at regulating or scheduling will receive a positive score (score of 1). If the TV is turned on in the morning and left on all during the day and night, regardless of what is on, a 0 is given. If the TV is left on during most of the child’s waking hours, a 0 is given. The Visitor needs to get a sense that the parent is regulating the amount of time the child is allowed to watch TV as well as making sure the shows are child appropriate. |
| 45 | Child can express negative feelings without harsh reprisal. | To most parents, “harsh” means physical punishment, and this is one acceptable interpretation for this item. A parent may still score positively (score of 1) on this item even if some punishment is given (for example, withdrawal of a privilege for a negative behavior) so long as the punishment is not excessive. However, if any negative expression on the part of the child leads to a big scene, overbearing lecture, or major withholding of a privilege, the score would be minus (score of 0). Suppose the negative behavior in question was a big temper tantrum with shouts of “I hate you” from the child. If the parent says, “I put him in the bedroom and say ‘Stay in there till you calm down,” ‘the score would be a 1. If, for the same expression of negative feeling, the parent says, “Well, I hate you too; now you just stay in your room for an hour,” the score would be 0. Other answers that would receive a 0 would be when the parent says, “I hit him hard,” or “I shake the tar out of him.” Remember that harsh reprisal includes mental (not just physical) punishment. |
| 46 | Child can hit parent without harsh reprisal. | In this case, harsh reprisal should be interpreted to mean physical punishment or other cruel disciplinary procedures. That is, score 1 if child can occasionally hit parent without being hit back, spanked, beaten, or berated. If, after you ask, the parent states that this has never happened, score a 1. |
| 47 | Parent introduces Visitor to child. | In many cases the child might already know the Visitor; however, the parent must still remind the child of the Visitor’s name. A formal introduction is not necessary for credit. A comment such as “You remember Mrs. Jones, don’t you?” or “Show Mrs. Jones the new book you got for your birthday” will receive credit. The object of the item is for the parent to help the child feel important by making the child aware of the Visitor’s name and by indicating that she has come to visit both of them and not just the parent. |
| 48 | Child has real or toy musical instrument. | Examples will include a piano, drum, toy xylophone, guitar, and radio (either real or toy). The mere presence of a musical instrument in the home is not enough. Child must be permitted to use it. The musical instrument does not have to belong to the child personally. |
| 49 | Child is taken on an outing by a family member at least every other week. | Family member can include anyone in the child’s immediate family (including older sibling as long as the person is over twelve years old) or extended family member such as an aunt, uncle, or cousin. These outings may include a shopping excursion, the barber shop, department store, picnic in the park, zoo, fast food restaurant, ice cream shop, etc. |
| 50 | Child has been on a trip more than 25 miles (40 km) during the past year. | The 25 miles (40 km) should be the distance from home, not the total roundtrip distance. Each Visitor will have to be the judge as to whether a trip recounted by the parent meets the distance requirements. |
| 51 | Child has been taken to a museum (or fair, exhibition, festival, temple) during the past year. | This is pretty must self-explanatory in that almost any type of museum (or other places listed in question) will do—a local art center, a children’s museum, a clock museum, natural history museum, or an art display even if held in a local bank or shopping mall. |
| 52 | Parent encourages child to put away toys without help. | Credit is given if the parent actually tries to get the child to pick up his or her toys after each play session or before going to bed rather than doing it herself because it is easier. Credit is given so long as the parent tries; it is not necessary that the child always comply. We are trying to get at the notion that the parent feels the child should be responsible for his/her own things. |
| 53 | Child eats at least one meal on most days with mother or father (or father figure). | (One parent families get an automatic 0.) This can be any meal during the day. The child must eat with the family either at the table or in a high chair pulled up to the table. In the cases of large families where perhaps not all the family can sit at the table at the same time or sit at the same table, credit is given if the index child eats with the parents. |
| 54 | Parent lets child choose certain favorite food products or brands at grocery store. | If the child is never taken to the grocery store with the parent, this gets an automatic 0. In order to receive credit, the child must express a desire for a product and be allowed to get it, not just select an item the mother has asked her to find. |
| 55 | Parent uses complex sentence structure and vocabulary. | If the mother makes an attempt at carrying on a regular conversation instead of just finding a way to answer all of the questions with “yes” or “no” or “I don’t know” and not giving explanation, this should be scored positively (score of 1). Lack of elaboration in responses or constant use of short sentences get a 0. |
| 56 | Child’s artwork is displayed some place in the house. | The artwork may be anything the child has made. Occasionally this can be observed, but it is often necessary to get at this through direct questioning. If the parent mentions that the child enjoys coloring and drawing during the discussion of toys, this presents an excellent opportunity to inquire about what she likes to do with her creations when she completes them or brings them home from child care or school. |
| 57 | No more than one instance of physical punishment occurred during the past week. | In this case the Visitor must take the word of the parent. Even if the parent states that this week was most unusual and the child received more than one spanking, the item should receive a 0. Definitions of “physical punishment” seem to vary considerably. Many parents consider it to be a spanking of any sort whereas others would consider restraint and shaking a child as physical punishment. If in doubt, take the parent’s definition. |
| 58 | Parent does not scold or yell at or derogate child more than once. | To derogate a child means to put them down (an example would be, “you are such a worthless child”). This is an observation item, and the time period referenced is the duration of the visit for the HOME observation. If you are at the home for another reason or, if you’ve completed your HOME observation and you have moved on to collecting other data, this time should be count toward observing this item (in other words, if you did not observe any scolding/yelling/derogating of child during the HOME observation time, you score this item as a 1; if you then do observe some of this behavior at a later time point, you do NOT change your score). In this item all scorable remarks must be made to the child; that is, the mother must tell the child that he is a bad boy and not simply tell the interviewer that the child is bad. If this occurs more than once during a visit, the item should be scored as a minus (0). |
| 59 | Parent does not use physical restraint during visit. | In a younger child, parental restraint might take the form of holding the child in the lap even though the child struggles to get down. Restraint for an older child might mean requiring him to sit in a chair to keep him out of the way, or jerking him back from handling items on a table, or pulling him away if he tries to climb on the interviewer’s lap. Also scored as a 0 are such acts as abruptly grabbing, shaking, or pinching the child—parental acts that are marginal from being classified as physical punishment but which nevertheless express considerable parental hostility to the child. Exceptions to this rule are instances where safety is a concern. For example, if a candle is lit and the child reaches his/her hand out toward the flame, the parent may grab the child’s arm/hand and pull it back. This would NOT be considered physical restraint, in this instance (so, safety reasons are an exception). |
| 60 | Parent neither slaps nor spanks child during visit. | This item goes hand in hand with #58. In this item the slaps and spanks must be in anger or as a reprimand for some wrong-doing. An affectionate pat on the bottom as the parent sends the child out to play does not mean the item should receive a negative score. |

**SRQ—Self-Reporting Questionnaire 20**

**I. Purpose**

The SRQ-20 will be administered to the mothers (or primary caregiver) in order to collect data on depressive symptoms. Given the known relation between depressive symptoms in the caregiver and child development, we are collecting this information in order to control for it in our statistical analyses.

**II. Materials**

SRQ Form, SRQ-20 SOP, clipboard, pen

**III. Methods**

The SRQ-20 will be administered by a Psychological Research Assistant (PRA) to the mother when the child is 5 years of age. The collection window for the assessment is ± 30 days (eg 5 years ± 30 days). We have included this ‘window period’ to make scheduling a bit easier. If the mother or primary caregiver is unable to be tested during this window period (for instance, if she is out of town), we will still collect this data as soon as she is able to be tested (for instance, when she returns). However, the date of test administration will be noted and therefore we will know that this data was collected at a slightly different time for that particular subject. If the date of the test administration is outside the ± 30 day window, a Protocol Deviation Form should be completed. Only the PRA and mother will be present during the administration of this questionnaire given the sensitive nature of some of the questions. This questionnaire can be used as a self-administered questionnaire or interviewer administered questionnaire. For purposes of consistency across the sites, we will employ the interviewer administered questionnaire technique.

This questionnaire is the same one that we administered between birth and 24 months of age. There are 20 items on the SRQ, each scored 0 or 1. A score of 1 indicates that the symptom was present during the past month while a score of 0 indicates that the symptom was absent. Therefore, the maximum score is 20.

The PRA will begin by introducing herself and explaining that she is asking these questions in order to get a sense of the mother’s mood over the last 30 days. In order to ensure that each mother receives the same instructions, the following instructions will be read by the PRA to the mother:

**“The following questions are related to certain pains and problems that may have bothered you the last 30 days. If you think the question applies to you and you had the described problem in the last 30 days, answer yes. On the other hand, if the question does not apply to you and you did not have the problem in the last 30 days, answer no. I am not allowed to discuss the items with you while we are completing the questionnaire. However, after all questions have been asked, I would be happy to go back and discuss any of the questions with you, if you’d like. Please do try to answer yes or no to every question that I ask you, even if it is approximate.”**

The PRA proceeds by asking every question on the questionnaire. After every other question, the PRA should remind the mother that the questions are asking about feelings/behaviors in the past 30 days. We want to make sure that the mother remembers that she is not reporting simply on the present. Once all of the questions have been asked and answered, the mother is free to discuss any of the questions with the PRA. This discussion time should not be used to change any of the previously given answers. Rather, this discussion time is used to allow subjects to feel that they are being listened to and serves as a way to discourage the mothers from insisting on discussion while the questionnaire is being administered.

Once the questionnaire is finished, if the PRA is concerned about the mother’s mental health (based on answers that she gave to the questions), s/he must immediately report this concern to his/her supervisor. The supervisor will then proceed with appropriate follow-up/referral.

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| **#** | **Question** | **Guidance** |
|  | Participant ID | Write the mother’s participant ID of the mother in the space provided at the upper left corner. |
|  | Study Researcher/  Nurse/Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker’s unique ID number here. |
|  | Today’s Date | Format DD/MMM/YY |
|  | Identity of primary caregiver | The mother is the person being interviewed for the SRQ-20. This question asks her to identify *who she considers* the primary caregiver of the study child.  Enter the code for the caregiver: 01=mother, 02=father, 03=grandmother, 04=grandfather, 05=aunt, 06=uncle, 07=other relative, 08=other nonrelative. |
| 01 | Do you often have headaches? | 00 = No; 01 = Yes |
| 02 | Is your appetite poor? | 00 = No; 01 = Yes |
| 03 | Do you sleep badly? | 00 = No; 01 = Yes |
| 04 | Are you easily frightened? | 00 = No; 01 = Yes |
| 05 | Do your hands shake? | 00 = No; 01 = Yes |
| 06 | Do you feel nervous, tense or worried? | 00 = No; 01 = Yes |
| 07 | Is your digestion poor? | 00 = No; 01 = Yes |
| 08 | Do you have trouble thinking clearly? | 00 = No; 01 = Yes |
| 09 | Do you feel unhappy? | 00 = No; 01 = Yes |
| 10 | Do you cry more than usual? | 00 = No; 01 = Yes |
| 11 | Do you find it difficult to enjoy your daily activities? | 00 = No; 01 = Yes |
| 12 | Do you find it difficult to make decisions? | 00 = No; 01 = Yes |
| 13 | Is your daily work suffering? | 00 = No; 01 = Yes |
| 14 | Are you unable to play a useful part in life? | 00 = No; 01 = Yes |
| 15 | Have you lost interest in things? | 00 = No; 01 = Yes |
| 16 | Do you feel that you are a worthless person? | 00 = No; 01 = Yes |
| 17 | Has the thought of ending your life been on your mind? | 00 = No; 01 = Yes |
| 18 | Do you feel tired all the time? | 00 = No; 01 = Yes |
| 19 | Do you have uncomfortable feelings in your stomach? | 00 = No; 01 = Yes |
| 20 | Are you easily tired? | 00 = No; 01 = Yes |

**Collection and Processing of Clinical Specimens**

**USV— Small Volume Urine Collection, Processing, and Transport**

1. **Purpose**

Urine specimens will be collected at 60 months of age.A small volume of urine (chlorhexidine free) will be collected from every enrolled child at 60 months of age in order to determine iodine concentration (in the future). The small volumes collections will NOT be preceded by Lactulose Mannitol ingestion and will be relatively simple to obtain. At 60 months, the 2 ml sample of chlorhexidine-free urine will be placed in a cryotube and frozen.

1. **Material**

Urine collection jars/cups (graduated 100 mL)

Pasteur pipets

2 ml cryotube (for iodine urine sample)

Cooler with ice packs

Min/Max thermometers (Fisher cat. #S90201)

Pre-printed specimen labels

Bath soap and paper towels and cleansing towelettes

Small Volume Urine Collection Form (USV) and SOP

**III. Methods**

At 60 months of age, ask the child to urinate on command and follow the below procedure:

* 1. The parent should wash her hands first, cleanse the child’s genital area with bath soap, rinse, dry and wipe with cleansing towelette, and allow the newly cleansed area to air-dry. DO NOT wipe over the area to speed drying. Once the area is cleansed and dried, the child should urinate into a collection cup. The child must **NOT** collect their urine from the toilet or any other receptacle. The contents of the cup should be transferred via pipet to appropriately labeled cryotubes.
  2. Enter the date of collection (DD/MMM/YY) and time of collection (hh:mm; 24 hour time scale). On the pre-printed labels, circle the U indicating that the sample is a urine sample. Please also indicate by circling if the urine sample is for iodine testing (I). The Sample ID will be entered once it is assigned in the laboratory.

The FW places the urine sample(s) into a ziplock bag containing absorbent toweling. All specimens are refrigerated (or kept cold in a cooler with a frozen ice pack) until transported to the research laboratory for analysis. For transport, urines are placed into a plastic cooler (labeled Biohazardous, BSL#2) containing a frozen ice pack. The cooler is securely sealed with tape and sent to the Laboratory, preferably on the same day as collection.

At the time of arrival at the research laboratory, the Sample ID is assigned to the urine specimen(s). If there are multiple aliquots, all aliquots are labeled with the same Sample ID. The lab technician should write the Sample ID on the existing labels.

Urine specimens should be stored at -80C until it is shipping or processing.

Collection information is completed on the Small Volume Urine Collection Form (USV).

**IV. Documentation**

All collections are noted on the Urine Collection and Receiving Form (USV) as mentioned above. Please ensure that the samples are labeled clearly. Any notes about spillage or other required information should be noted in the Observations field.

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| # | Question | Guidance |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| **Urine collection (Chlorhexidine Free)** | | |
| 01 | Fieldworker ID | Enter the fieldworker’s unique ID number who collected the urine sample. |
| 02 | Date | Enter the date of collection (DD/MMM/YY) of the urine sample. Should be collected during the monthly surveillance visit within the window DOB -2/+7 days. |
| 03 | Month of collection (scheduled) | Enter the scheduled month of collection. Possible answers: 12, 18, 21 (for BMMI only), 24, 36, 60. |
| 04 | Is this child in the BMMI cohort? | Indicated whether this child is in the BMMI cohort. 00=No; 01=Yes, NA=Not Applicable. Mark NA for 60m assessment |
| 05 | Total Urine Volume | Measure the volume of collected urine in a graduated container. Record the total volume in mL.  **For children in the MAL-ED cohort (24, 36, and 60m): at least 2ml of urine should be collected**  **For children in the BMMI cohort (12, 18, 21, 24m): at least 2ml of urine should be collected**  **For children in the BMMI cohort—At month 24, at least 4ml of urine should be collected (2ml for MAL-ED and 2ml for BMMI).** |
| 06 | Time of departure from field | Enter the time of departure from the field (either the site of urine collection or the field laboratory if applicable). Enter the time using the 24 hour time scale (HH:MM). |
| **Urine transport and receiving** | | |
| 07 | Lab Technician ID | Enter the Lab Technician’s unique ID number who processes or receives the urine sample, if different from the FW who collected the sample. If not applicable, strike through the first box and enter NA. |
| 08 | Time of arrival at research lab | Enter the time of arrival at the Research Laboratory. Enter the time using the 24 hour time scale (HH:MM). |
| 09 | Sample ID | Write the 9 digit Sample ID. |
| 10 | Was urine collected for MAL-ED iodine testing? | Indicate whether urine was collected for iodine testing (should be collected at 24, 36 and 60m).  Possible answers: 00=No; 01=Yes; NA=Not Applicable |
| 11 | Number of aliquots for BMMI | Enter the number of aliquots collected for BMMI. Enter the number as 2 digits. If no urine was collected for BMMI, enter NA. |
|  | Observations | Record any observations here. |

**BCH—Blood Collection SOP**

**Purpose:**

A blood sample will be collected at 60 months of age and archived for future assessment of micronutrient status. Field workers and a nurse phlebotomist should work as a team to collect these samples in the subject’s home or in the clinic (depending on site). Approximately 6 mL of blood will be obtained from participating children at 60 months of age.

Information regarding blood collection will be recorded on the Blood Collection and Hemoglobin Form (BCH). See details on reporting at the end of this SOP.

**Collection Window:**

Because time of day can affect nutrient values, we want to standardize the time of day for blood collection. Based on a survey of the sites, it seems that **morning blood collection (7-11 am)** is possible but may need to be adjusted if moms are working or child is in school. Time of day will be noted on the collection sheet.

The window for collection of the blood samples should be **DOB +/- 2 days**. However, due to the possibility that the child may be ill (see illness criteria that would delay collection of blood sample, below) we will permit this collection for up to 12 days after the DOB (**window = DOB -2/+12 days for a total of 15 days**). If the extended time is needed, fill out a protocol deviation form and explain the illness or reason that prevented collection in the regular window.

**Illness Criteria that would delay blood draw:**

* Child is listless, unable to perform normal activity
* Axillary temperature > 37.5 degrees C
* ALRI (pneumonia)
* Some dehydration - demonstrates 2/4 of the following criteria:

(1) restless, irritable, (2) sunken eyes, (3) drinks eagerly, thirsty, (4) skin pinch goes back slowly. Child must be rehydrated before blood is taken. Child must be referred to a clinic for rehydration.

* Bloody stool (caregiver reported, fieldworker observed)
* Frequent diarrhea (>8 loose stools in previous 24 hour period)
* Persistent diarrhea (child meets definition of diarrhea for 14 or more days)

**Currently, the samples will be collected and archived as the funding does not cover analysis of the samples. However, in the future, we hope to obtain funding for the following assays:**

* **Hemoglobin (this is funded and will be done in the field)** - 1 drop of whole blood for hemoglobin assay (HemoCue to be done in the field). The drop can be obtained from the tubing when using butterfly syringe system.
* **Pending funding, the following assays will be done on plasma. They are listed in decreasing order of importance, if the sample is limited.**

1. Plasma ferritin

Plasma zinc

Plasma transferrin receptor

Plasma Alpha-1-acid glycoprotein (AGP)

C-reactive protein

Plasma retinol

* **Lead** - 0.5 mL heparinized **whole blood** will be required for lead determination. This sample may be obtained from the tubing if butterfly system is used, in addition to the drop needed for the HemoCue cuvette. If enough blood is not available from tubing, transfer 0.5 mL of whole blood from syringe to a heparinized vacutainer tube (tan stopper) for lead.

**Other considerations:**

* **It is vitally important that all the materials -** **syringes, tubing, needles, stoppers, vacutainers, and gloves - be certified by the manufacturer for use for trace minerals or follow best practices, which are detailed in the table below.**
* For determination of plasma retinol, it is important that light exposure be minimized. Plasma is more sensitive to light than is whole blood, and available means to limit light exposure should be used. In the field, this includes drawing blinds/shades, turning off existing lighting, and covering the collected sample tube with aluminum foil prior to putting on cold packs.
* In the laboratory, special lighting should be used, amber crepe paper can be placed over the lights, or work can be scheduled during daylight hours and in a room with indirect lighting to enable work without compromising the sample. The cryovials in which the plasma is stored for retinol analysis (after centrifugation of whole blood) should also be covered with aluminum foil prior to freezing.

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| Item | Requirements | Options |
| Gloves | Non-latex, no talc, no rubber  Polyethylene—yes!  Nitrile – ok based on track record among zinc labs | Fisher Scientific: ([www.fishersci.com](http://www.fishersci.com))  Cat.#19-166-709: Cardinal Health Nitrile gloves (medium) |
| Tourniquets | Latex-Free | VWR: ([www.vwr.com](http://www.vwr.com))  Cat.#VT367203: BD Vacutainer Latex-Free Stretch Tourniquets, BD Diagnostics |
| Tubes | Certified for trace mineral analyses | Becton Dickinson: (<http://www.bd.com>)  Cat.#368381: 6 mL tube with K2EDTA for plasma (royal blue stopper)  Cat.#367855: 3 mL tube with K2EDTA for lead (tan stopper),  Kendall Health Care: (<http://www.kendallhealthcare.com/kendallhealthcare/pageBuilder.aspx?webPageID=0&topicID=75982&xsl=xsl/productPagePrint.xsl>  Cat. #8881307022: 7 mL tube with NaEDTA for plasma (royal blue stopper) |
| Transfer Needles | 21 gauge recommended to reduce hemolysis  Stainless steel, siliconized or polypropylene | Becton Dickinson: (<http://www.bd.com>)  Cat.#305764 – 21 g. Eclipse safety needle  Cat.#305915 – 21 g. SafetyGlide needle |
| Butterfly blood collection system | According to Dr. Krebs, Terumo Medical is the only vendor they have found to produce butterfly systems free of zinc. Experience amongst other researchers and the manufacturers is that the tubing is PVC and not likely to be a source of zinc contamination – so they might be fine. | Becton Dickinson: (<http://www.bd.com>)  Cat.#367342 – 23 g. Vacutainer Push Button Blood Collection Set  Terumo Medical Products: (<http://www.terumotmp.com/ProductDetails.aspx?categoryId=6&productId=298>) |
| Cryovials | Tubes with screw cap without rubber O-ring | Fisher Scientific: ([www.fishersci.com](http://www.fishersci.com))  Cat.#12-565-168N  ThermoScientific/Nunc: ([www.thermoscientific.com/en/product](http://www.thermoscientific.com/en/product))  Cat.#377224 - 1.0 mL Freestanding conical cryotube |
| Labels |  | Laser cryo-babies: ([www.divbio.com](http://www.divbio.com))  Cat.#CRY-0500 Cryo-Babies |
| Sharps Disposal Containers |  | VWR: ([www.vwr.com](http://www.vwr.com))  Cat#.89002-264 PG2 DOT-Compliant Sharps Disposal Containers, Covidien |

**Specimen Collection:**

Depending on the skill level of the phlebotomist, different systems for blood collection may be available. Thus, while both the equipment list and the protocol are written for a butterfly needle system, this is written only as a guide. Each site will make decisions and order supplies as appropriate for their site, following the principles laid out here.

**Blood Collection Kit - store items listed in a box or container to carry to the field:**

1. Blood collection SOP (SCF), SCF Form, pen, lab marker
2. Alcohol hand wash toweling
3. Tourniquets, latex-free
4. Disposable, trace element free, polyethylene or nitrile gloves, free of talc and other coatings - no rubber!
5. Alcohol wipe/swab
6. Syringes 5-10cc (polyethylene syringe and plunger – no rubber)
7. 21-23 g. Butterfly blood collection system
8. 6-7 mL trace element-free vacutainer tube with anti-coagulant (ie. BD royal blue stopper)
9. Hemoglobin Cuvette (HemoCue cat.#651200XX)
10. 3 mL vacutainer tube with K2EDTA anticoagulant for lead determination (i.e. BD tan stopper)
11. Single sample transfer needle (19-21g)
12. Aluminum foil (for wrapping tubes for light sensitive retinol analysis)
13. Cotton ball or 2X2 gauze
14. Adhesive bandage
15. Tube racks
16. Zippered plastic baggie
17. Sharps disposal container for needles and syringes
18. Cooler with blue ice packs and tube rack for blood tubes
19. Min-Max Thermometer (optional, for monitoring cooler temperature)

**Procedure Summary:**

1. FW collects schedule of venipuncture list from data management team or field supervisor
2. Prepare and restock Blood Collection Kit
3. Place frozen blue ice packs in cooler (optional: add thermometer to cooler)
4. Phlebotomist and 1-2 FWs transport Blood Collection Kit and cooler to the subject’s home (or clinic)
5. FW introduces the phlebotomist to the mother/guardian and child
6. FW asks mother/guardian about child’s health and records the child’s temperature
7. Phlebotomist and FW wash hands with the alcohol toweling, put on trace mineral free gloves
8. Phlebotomist prepares, labels and organizes one set of blood collection materials
9. Phlebotomist collects blood and hands the syringe to the FW. Phlebotomist continues to attend to the child and applies the adhesive bandage.
10. FW transfers a drop of blood to the HemoCue cuvette from the butterfly tubing and places cuvette into HemoCue reader.
11. FW places the transfer needle on the syringe and adds 5 mL of blood to the royal blue stoppered vacutainer tube and 0.5 mL from the butterfly tubing is added to the 3 mL tan stoppered vacutainer tube for lead analysis.
12. FW inverts vacutainer tubes, records HemoCue value, labels tubes, places tubes in ziplock baggie and puts baggie in cooler with blue ice.
13. FW cleans area of all venipuncture supplies, places used syringe and needles into sharps disposal container and wipes hands and all surfaces with alcohol toweling.

**Notes:**

**A. Introduction to caregiver**

1. Field worker should introduce the nurse / phlebotomist to the caregiver. Be warm and friendly, establish eye contact, and show concern about the child’s health and comfort. You first should instill a trust and confidence in parent and child.
2. Ask about the child’s past experience with blood drawing.
3. Ask about the child’s current health and take child’s temperature if fever is suspected (>37.5C). Postpone if child is ill as determined by the Illness Criteria.

**B. Positions for restraining a combative child:**

1. The physical restraint of the child by a supportive parent is the best situation. The parent holds the child in their lap and wraps their legs around the child’s so that the child cannot kick the phlebotomist.
2. In the horizontal position, the child lies supine, with the phlebotomist on one side of the bed and the parent on the other. The parent gently but firmly leans over the child, restraining the near arm and body while holding the opposite extended arm securely.
3. In the event the child cannot be restrained without potential physical injury, discontinue attempt and notify the research staff.
4. FW may help distract child.

**C. Illustrative Antecubital Fossa Vein Technique**

At 5 years of age, the antecubital fossa vein is best for blood draw.

1. Wash hands and put on gloves.
2. Identify the child.
3. Prepare and organize venipuncture equipment:

a. Label SCF form

b. Attach a 21-23g butterfly needle collection set to a 5-10 ml syringe. Keep equipment out of the view of the child. A 21g is preferred to a 23g to reduce chance of hemolysis but it is at the discretion of the phlebotomist.

1. Gather following specimen collection containers and label them with the child’s PID as appropriate.

* One 6-7 mL trace element free plasma tube (5 mL whole blood)
* One HemoCue cuvette for drop of blood for Hemoglobin
* One 3 mL tan top tube (Becton Dickinson) for whole blood for lead determination (or other LiHeparin tube recommended for lead)

1. Select the arm that has easily visible veins. Application of a tourniquet promotes venous distension.
2. Lower the extremity below the level of the heart.
3. Lightly palpate for a vein. Apply warm compresses, if needed, to increase vasodilation.
4. Disinfect the site with 70% isopropyl alcohol swab in concentric circles. Let alcohol air dry.
5. Fix the vein by applying pressure to skin over the vein, approximately two inches below venipuncture site.
6. Approach the skin with needle bevel uppermost at an angle of 35-45 degrees. When the needle has penetrated the skin, realign it with the vein and reduce the angle to about 15 degrees to the skin.
7. As soon as blood appears in the tubing of the butterfly, pull the syringe plunger to fill with blood. Make sure not to exceed maximum daily blood volume (5 mL). Release the tourniquet.
8. Place clean gauze or piece of cotton wool over the needle as it is gently withdrawn and hold direct pressure on the puncture site until bleeding stops. Apply an adhesive bandage.
9. Engage Safety-Lok feature on butterfly needle and hand the syringe and butterfly tubing to the FW.
10. FW disconnects butterfly from the syringe and extracts the blood from the tubing for the cuvette for the HemoCue. Use one drop of blood contained in the collection tubing (between the butterfly needle and the syringe) for the HemoCue assay of hemoglobin. This assay is done immediately and the value obtained recorded on the SCF Blood Collection Form. Refer to the HemoCue SOP for further instructions on this assay. Discard needle/tubing into a Sharps container.
11. Connect 19-21 g single sample transfer needle to syringe to add blood to the vacutainer tube.
12. Without removing vacutainer tube from tube holder, insert needle into tube and fill appropriately (for both plasma (royal blue top) and whole blood (tan top) tubes):

Note: Let vacuum draw the blood into the tubes. Angle needle against the inside of the vacutainer wall. Never force blood into the tubes. Dispose syringe and needle into Sharps container. Do not disconnect or recap the needle.

1. Gently invert additive tubes 8-10 times and label tubes with:

* Child’s PID number
* Research record number
* Date
* Time of draw
* Blood collector’s initials

1. Wrap vacuum tube in aluminum foil to protect from light (for retinol analysis)
2. Report collection of child’s sample on the Blood Collection Form (SCF).
3. **NOTE:** **the sample must immediately be placed in cooler with cold packs** **or be refrigerated. Keep on cold packs or refrigerated until centrifugation, preferably within 2 hours.**

**PERSONAL INJURIES**

**CAUTION: When working with needles, lancets, and other “sharps”, be careful not to prick your finger. All injuries incurred with sharps no matter how minor, must be reported immediately so appropriate follow-up procedures can be initiated if necessary. Wash with soap and water. Apply pressure to wound to curtail bleeding if necessary and apply a bandage. Report any injury involving sharps to a Study Coordinator or Principal Investigator and report to your local Casualty Department for treatment and evaluation for post-exposure prophylaxis.**

**D. Reporting (Blood Collection and Hemoglobin Form, BCH)**

Study Researchers/Phlebotomists will record information regarding blood collection on the Blood Collection and Hemoglobin Form (SCF). Please see the table below for assistance. There is space provided at the end of the form to provide additional information, observations and notes.

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
|  | Participant ID | Write the participant ID in the space provided at the upper left corner. |
| 01 | Phlebotomist ID | Enter the Study Researcher/Phlebotomist’s unique ID number, who will perform the blood draw. |
| 02 | Month of blood draw? | 7 month = 01;  15 month = 02;  24 month = 03;  36 month = 04;  60 month = 05 |
| 03 | Date of collection: | DD/MMM/YY |
| 04 | Time of collection | Enter the time that the Study Researcher/Phlebotomist visited the home to collect the blood sample. If no sample was collected, this is the arrival time of the visit. |
| 05 | Last meal/breastfeed | Enter information about the last meal or breastfeed given to the study participant. If breast milk only, enter 01. If food or snack only, enter 02. If food or meal only, enter 03. If both food and breast milk, enter 04. |
| 06 | Time since last meal/breastfeed | Enter the time since the study participant last had a meal or was breastfed. If the last meal or breastfeed was within the last 30 minutes, enter 01. If it was within the last 2 hours, enter 02. If it was greater than 2 hours ago, enter 03. |
| 07 | Tube 1 with **up to** 5mL blood collected? | Yes=01, No=00 |
| 08 | Hemocue filled (one drop)? | Indicate if one drop of whole blood was used for hemoglobin determination via Hemocue. If the Hemocue was filled, enter 01. If not, enter 00. See the relevant Hemocue SOP (HEM). |
| 09 | Hemocue Hb level reading | Enter the hemoglobin measurement (##.# g/dL). See the relevant Hemocue SOP (HEM). |
| 10 | Tube 2 with 0.5 mL blood collected? | Yes=01, No=00, NA=Not Applicable |
|  | Observations/Notes | Enter any relevant observations or notes here. If, for example, the child was ill and the Study Researcher/ Phlebotomist was unable to obtain a blood sample, explain in this section. |

**HEM—HemoCue Assay SOP**

**PURPOSE**

Quantitative determination of hemoglobin in blood using the HemoCue HB201 analyzer.

**PRINCIPLE**

The blood sample is drawn into a microcuvette by capillary action and is mixed with sodium deoxycholate reagent deposited on its inner wall. This disintegrates the erythrocyte membranes releasing hemoglobin. Sodium nitrite converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin which then combines with azide to form azidemethemoglobin. The microcuvette is then placed in HemoCue Hb analyzer in which the transmittance is measured and the hemoglobin level calculated. The HB 201 requires HB 201 specific cuvettes.

**SCOPE**

This Standard Operating Procedure applies to all point of care testing for hemoglobin performed by the Study Researcher / Nurse / Fieldworker who has been trained and is competent in performing this test.

**STANDARD PRECAUTIONS**

Observe Standard Precautions when collecting and handling blood samples and performing the test. Wear gloves to protect against exposure to bloodborne pathogens.

**SPECIMEN COLLECTION**

Specimen: See SCF Blood Collection SOP for collection of venous blood

Minimal volume required: 5 ul (one 4-5 mm drop of blood)

Test is performed immediately

**EQUIPMENT**

1. HemoCue HB 201 analyzer

**MATERIALS**

1. HemoCue Hb 201 microcuvettes
2. Butterfly blood collection system (see SCF SOP)
3. Non-fraying swab (lint-free)
4. 4AA batteries if main power is not available
5. Biohazard disposable container

**STORAGE REQUIREMENTS**

Microcuvettes - 15 – 30ºC

*Microcuvettes are moisture sensitive so it is important that the cap of the vial is closed properly between uses. Unused microcuvettes must be kept in vials. DO NOT REFRIGERATE.*

**QUALITY CONTROL**

**1. HEMOCUE Hb ANALYSER PERFORMANCE CHECK**

The HemoCue 201 analyzer has an internal electronic “SELF TEST” which automatically verifies the performance of the optronic unit of the analyzer. This test is performed at regular intervals when the analyzer is on. Each day of use, analyzer performance must be checked prior to testing patient samples.

**Frequency:** Each day of use prior to patient testing.

**Procedure:**

1. Pull cuvette holder out to its loading position.
2. Press and hold the left button until the display is activated.
3. The display shows the version number of the program followed by an hourglass then “Hb”. During this time the analyzer will automatically verify the unit.
4. After 10 seconds the display will show 3 flashing dashes and the HemoCue symbol which indicates the analyzer is ready for use.

**Acceptable Results**: HemoCue display shows the HemoCue symbol indicating the unit is ready for use.

**Corrective Action:** Repeat Performance Check. If still unacceptable do not use analyzer for patient tests. Arrange for repair or replacement of unit.

**Documentation:** Enter date, initials of person performing test and “OK” on HemoCue Analyzer QC Record sheet.

**2. HEMOCUE 201 ANALYSER MAINTENANCE**

Periodic cleaning of HemoCue 201 Analyzer is recommended by the manufacturer.

**Frequency**: Once per week

**Performed by:** Lab staff

**Materials Required:** Alcohol, HemoCue Cleaner Swab

**Procedure:** (Refer to drawings on Page 28 of Operating Manual)

1. Turn off the analyzer.
2. Pull the cuvette holder out to its loading position.
3. Use a pointed object to carefully depress the small catch in the upper right corner of the cuvette holder.
4. With the catch depressed, carefully pull the cuvette holder in the direction in which the handle of the cuvette holder is pointing.
5. Clean the cuvette holder with alcohol.
6. Insert the HemoCue Cleaner swab into the opening of the cuvette holder.
7. Pull out and push in 5-10 times. If the swab is stained, repeat with a new swab. Cleaning is complete when swab comes out unstained.
8. Wait 15 minutes before using the analyzer.

**Documentation:** Record number of analyzer, date, initials of person cleaning the analyzer on the appropriate QC Record Sheet.

**3. Internal QC: Control Reagents**

**Control:** Manufacturer’s Control Reagents: Low, Normal, and High. ***Do not use beyond expiry date***.

**Frequency:** Weekly prior to patient testing and new lot numbers

**Procedure:**

1. Mix Control Reagent thoroughly.
2. Perform test in the same manner as for patient blood testing.

**Acceptable results:** Within acceptable range stated on reagent control

• Any QC “out of range” must be recorded and the follow-up action indicated.

◦ If the initial QC is out of range, repeat the same QC and document the corrective action taken.

◦ If the second QC determination is out of range proceed with problem solving.

◦ Document all action(s) taken.

Unless or until the QC is performed as within range, no patient testing may occur

**Corrective Actions**:

1. Check that microcuvettes and Reagent Control have been properly stored and are not expired.
2. Check cuvette for air bubbles and if present repeat test with new microcuvette.
3. Clean optronic unit with HemoCue Cleaner and repeat test.
4. If still unsatisfactory, do not perform patient testing and arrange for repair or replacement of analyzer.

**Documentation**:

1. Record date, lot# of Control Reagent, expiration date of Control Reagent, initials of person performing QC and the results obtained on the HemoCue Test QC Record sheet.
2. QC results will be reviewed monthly by Laboratory Supervisor or designee.
3. All Corrective Action should be documented and reviewed.
4. Submit completed QC sheets to Biotechnology Laboratory Supervisor.

**External QC:**

1. Splitting samples among different operators within the laboratory times/year (operator proficiency) twice yearly.
2. Comparison of 2 test methodologies : Hemocue vs reference instrument (on site automated hematology analyzer) Twice yearly with an allowable difference of 7.5%

**TEST PROCEDURE**

***Must be performed within 10 minutes of blood collection***.

1. Pull the cuvette holder out to its loading position. Press and hold the on/off button until the display is activated.
2. Take the microcuvette out of the package. If microcuvettes from a vial are used, replace the cap immediately after the microcuvette has been removed.
3. Disconnect butterfly from the syringe (see SCF procedures above) and extract the blood from the tubing for the cuvette for the HemoCue. Use one drop of blood contained in the collection tubing (between the butterfly needle and the syringe). Hold the microcuvette opposite the filling end and bring into contact with blood sample, allow the cavity of the microcuvette to fill completely by capillary action.
4. When completely filled, wipe off the outside of the microcuvette with a clean lint – free tissue, being careful not to touch the open end of the microcuvette.

***If air bubbles are seen in the optical eye, discard the microcuvette and refill new microcuvette. Small air bubbles around the edge do not influence the result.***

1. Place filled HemoCue Hb 201 microcuvette in the cuvette holder for the analyzer.
2. Push the cuvette holder to its measuring position within 10 seconds after filling.
3. After 10 sec HemoCue Hb 201 analyzer will find the steady state of the chemical reaction and the result will appear in the display. Record result on SCF form.
4. Pull the cuvette holder out to its loading position and discard used microcuvette in a biohazard waste container.

**INTERPRETATION OF RESULTS**

Hemoglobin value is read directly from HemoCue Hb 201 analyzer in g/dL (grams per deciliter)

Results above 25.6g/dL are displayed as **HHH** or **over-range.**

**HHH/Over-range Results** – Repeat test.

Reasons for abnormally high results include:

Expired microcuvettes

Contaminated optical eye of microcuvette

Improper mixing if Control Reagent

Air bubbles in the microcuvette

Optronic unit is dirty.

**RECORDING/REPORTING RESULTS**

1. Enter test result on the SCF form in the space provided (#8).
2. A hemoglobin of **<5 g/dL** is considered a **critical result**.
3. Report critical results directly to your supervisor who should report to a physician.

**TEST LIMITATIONS**

1. Measurement of hemoglobin should be made as soon as possible after the blood has been drawn into the microcuvette. If the readings are made after 10-minutes of filling the microcuvette inaccurate results may be obtained.
2. Mixing for too long a period can produce unreleased oxygen pressure and viscosity that may cause falsely high results.
3. Failure to hold the microcuvette by the filling end may stain the optical eye and give inaccurate results.
4. Failure to wipe excess specimen from the outer surface of the optical eye may give inaccurate results.

**REFERENCES:**

Package insert, HemoCue HB 201, HemoCue AB, Angelholm, Sweden.

Operating Manual, HemoCue HB 201, Version 901702/020828. HemoCue AB, Box 1204, Angelholm, Sweden.

Paiva, AA, etal. 2004. Comparison between the HemoCue and an automated counter for measuring hemoglobin. Rev. Saude. Publica. 38:585.

**BRF—Blood Processing, Transport and Storage and Blood Receiving**

Risk of contamination is high in the laboratory unless proper procedures are maintained. Trace mineral free supplies must be used, and care must be taken to reduce exposure of the sample to air, dust, water, hair, and skin cells from the lab technician. Samples should be processed in laminar flow clean rooms, laminar flow hoods, or otherwise clean, dust and smoke free. Laboratory technicians should use trace element free polyethylene gloves, free of talc or other coatings.

To the extent possible, samples should be processed in a lab with indirect lighting. There is special lighting that can be used, or the lab can put amber crepe paper over the lights to enable work without compromising the sample for retinol determination, or to work in natural (indirect) light. If such safe lighting conditions are not possible, the sample should be covered in aluminum to shield it from the light.

Information on received blood samples will be recorded on the Blood Receiving Form (SRC). See details on reporting at the end of this SOP.

Processing, Transport, and Storage Materials

1. Centrifuge
2. Trace mineral free transfer pipet (e.g. [www.fishersci.com](http://www.fishersci.com) Cat.#13-711-31)
3. 6-1mL trace element free cryotubes for storage (ie. [www.fishersci.com](http://www.fishersci.com) Fisher Cat. #12-565-168N ThermoScientific/Nunc cat.#377224 - 1.0mL freestanding conical cryotube
4. Cryovial boxes
5. Refrigerator (large size)
6. Coolers with cold packs to transport blood to the laboratory
7. Ultra-low freezer (-70 C) with UPS system
8. Labels (eg. Laser cryo-babies and cryotags [www.divbio.com](http://www.divbio.com) Cryo Tags .94 in.x 0.5 in. cat.# CRY-0500 )

**Processing the sample:**

The sample should be transported on cold packs to the laboratory for processing. Upon arrival at the laboratory, the sample collected in the royal blue top should be centrifuged at 2000 x g for 10-15 minutes. Approximately 2.5 mL of plasma will be aliquoted into cryotubes (about 0.5mL per tube, see below).

It is important that the sample be centrifuged and processed as soon as possible. If not possible, the sample should be refrigerated until processing. The time the sample is processed will be logged so that we can examine the effect of time from blood draw to processing on plasma zinc concentrations (provided we get funding to do so in the future). We expect that zinc will leave the RBC and elevate the zinc concentration and so it is important that delay between blood draw and processing be minimized.

After centrifuging (note appearance of centrifuged blood in figure below), use a trace mineral free transfer pipet to aspirate the plasma fraction, being careful not to aspirate the buffy coat at the interface of the plasma and the red cells, and aliquot the plasma into cryovials as indicated below. NOTE: Be careful not to disturb cells when drawing plasma into a pipette. If cells mix with the plasma, re-centrifuge the sample to insure obtaining the maximum amount possible. When using pipettes, avoid drawing buffy coat or red cells into the bulb. The cryovials need labels appropriate for low temperature exposures (see table). How the sample is then aliquoted for storage at -70 degree Centigrade depends on the study site.

**Plasma:**

Aliquot plasma: listed in decreasing order of importance if the blood sample is limited.

1. 2 cryotubes for micronutrients (0.6 mL each at JHU, other sites may need more)
2. 2-3 cryotubes for any remaining plasma

**Sample collected in tan top tube (for lead analysis):**

1. **Sample should** **NOT BE CENTRIFUGED**
2. Transfer whole blood sample to a cryovial

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Figure 1. Appearance of Blood Samples and Recovery of WBCs (buffy coat)** | | | | | | Whole blood in the collection tube | Blood after centrifugation | WBCs and RBCs after plasma removal | Top view of the WBCs (buffy coat) | Top view of sample after WBC removal | | Tube_1 | Tube_2 | Tube_3 | Tube_4 | Tube_5 | |

**Long term storage:** Place the storage vials containing plasma and whole blood immediately in a vial box and store in a freezer at -70°C.

**Transport overseas:** Currently, we are not funded to ship the samples anywhere for processing nor are we funded to analyze the blood samples. As such, each lab will store the samples until further funding is obtained.

**Reporting (Blood Receiving and Processing Form, BRF):**

Laboratory Technicians will record information regarding the receipt of blood samples on the Blood Receiving Form (BRF). Please see the table below for assistance. There is space provided at the end of the form to provide additional information, observations and notes.

|  |  |  |
| --- | --- | --- |
| # | Question | Guidance |
|  | Participant ID | Write the participant ID in the space provided at the upper left corner. |
| 01 | Technician ID: | Enter the Laboratory Technician’s unique ID number, who received the blood sample. |
| 02 | Date of Collection: | Enter the date of collection (DD/MMM/YY) of the sample |
| 03 | Date of Arrival: | Enter the date of the arrival (DD/MMM/YY) at the laboratory. |
| 04 | Time of Arrival: | Enter the time that the blood sample(s) arrived in the laboratory (HH:MM) |
| 05 | Tube 1 with **up to** 5mL blood received? | Yes=01, No=00 |
| 06 | Tube 2 with 0.5 mL blood received | Yes=01, No=00, NA=Not Applicable |
| 07 | Sample ID: | Enter the Sample ID number assigned to the blood samples. Note that both samples will receive the same Sample ID number. |
| Concerning Tube 1 with up to 5 mL blood: | | |
| 08 | Date of centrifugation | Enter the date of centrifugation (DD/MMM/YY) at the laboratory |
| 09 | Time of centrifugation | Enter the time that the blood sample(s) were centrifuged in the laboratory (HH:MM) |
| 10 | Number of plasma aliquots | Enter the number of aliquots (cryovial tubes) for the plasma (0 – 9) |
| Concerning Tube 2 with 0.5 mL blood (Do Not Centrifuge): | | |
| 11 | Was whole blood transferred to cryovial for storage | Enter whether or not whole blood was transferred from tab top tube to a cryovial for storage. Enter 01 if yes and 00 if no. |
|  | Observations/Notes | Enter any relevant observations or notes here. |

**SFC—Stool Collection, Processing and Transport to the Laboratory**

1.0 **Purpose**

This protocol describes the procedure for the 60+ month surveillance stool specimen collection from MAL-ED research subjects enrolled in the cohort and timely transport to the laboratory for further processing. This specimen is collected in order to perform the Taqman Array Card for enteropathogens, examine for helminths microscopically and archive for future tests. Effort should be made to maintain the cold chain and freeze archived aliquots at -70 to -80 C as quickly as possible. The basic SFC data collection forms and database are unchanged, except we wish to record the time the stool aliquots were frozen and there will be "NA" for some of the old questions that no longer apply. Documenting time into freezer may be very helpful for future studies such as BMMI, thus the SRF adds Q24: Time to freezer

2.0 **Responsibility**

Field coordinator is responsible for ensuring that standard methodology described here is followed in stool collection and transport.

3.0 **Materials**

3.1 Stool specimen (2 grams)

3.2 Pre-printed PID labels (4 plus 1 extra)

3.3 Cotton-tipped wooden stick

3.4 Wide-mouthed plastic container suitable for collecting stools

3.5 Wooden spatula

3.6 Vial with 10 ml of 10% buffered formalin

3.7 Frozen ice packs

3.8 Cold box

3.9 Tube rack

3.10 Disposable latex gloves

3.11 Sealable plastic bags

3.12 Plastic spoon

3.13 Pen

3.14 Stool Field Collection Form (SFC)

4.0 **Collection Procedure for 60 Month surveillance Stool.**

4.1 Collect a 60 month stool, if the child is already older then 60 months collect a stool as soon as feasible (any time up to 72 months or even later is fine for this). We would like these to be "surveillance/monthly" stools i.e. **not diarrheal/not within 7 days of a diarrheal episod**e, because the purpose is to look at worms, growth, and cognitive scores

4.2The samples can be collected on the anniversary of the child’s birth (+/- 2 days) in keeping with the 1-24 month protocol. However, the field worker may visit the home AT ANYTIME to collect the stool sample.

4.3 Inform child’s primary caretaker one day before planned stool collection and request caretaker to collect the first available fresh stool sample from the child on the morning of the planned visit.

4.4 The mother should be provided with the labeled stool container, cold box, ice packs, gloves, plastic spoon, and 2 plastic bags the evening before planned stool collection. There should be enough ice packs in the cold box to keep it cold for up to 16 hours.

4.5 Instruct the caretaker to use the plastic spoon to collect 3-4 spoons of stool and place it in the stool container, close the lid tightly, and place the container in the plastic bag

4.6 Caregivers should be instructed about the urgency of collection of a fresh sample and getting that sample into the cold box

4.7 When a Study Researcher / Nurse / Fieldworker arrives at the home and a sample has already been collected by the caregiver, ask the caregiver what time the sample was produced and record that time (on SFC, question #4). In most cases this will only be an estimate.

4.8 Document if the sample was in a cold environment on the requisition CRF. Also document if specimen is acceptable (estimated quantity, lid closed, and no leakage)

5.0 **Temporary Storage and Transport Procedures**, **Field** **Processing**

5.1 Label the original stool container, formalin vial with pre-printed PID

5.2. Sites may elect to place 1 gram of stool using the wooden spatula in 10% formalin vial in the field. SFC The preserved stool will be used for microscopic examination for ova and parasites. The preferred Formalin to stool ratio is 3:1.

5.3 Place the original stool container with the remaining stool, and the stool in formalin vial in the cold box and transport with fresh ice packs to the laboratory for further processing.

**6.0 Reporting**

6.1 Record information on Stool Field Collection Form (SFC).

|  |  |  |
| --- | --- | --- |
| # | Question | Guidance |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| 01 | Date of visit | Enter the date of the visit (DD/MMM/YY) by the Study Researcher / Nurse / Fieldworker. |
| 02 | Time of visit | Enter the time that the Study Researcher / Nurse / Fieldworker visited the home to collect and pick-up the stool sample. If no sample was collected, this is the arrival time of the visit. |
| 03 | Was a stool sample collected? | Indicate whether or not a stool sample was collected. If yes, go to question #4. If no, go to question #8. |
| 04 | Time stool sample was produced | Enter the time (24 hour time scale, HH:MM) that the stool sample was produced. If this time is not available, estimate if time cannot be confirmed by care giver. |
| 05 | Time stool sample was preserved in Cary Blair | Enter the time that the stool sample was preserved in Cary Blair. |
| 06 | Type of stool collected | Indicate whether the stool sample was a monthly, diarrheal, monthly recollection, or diarrheal recollectionThis should only be M1 for the 60m sample |
| 07 | Month of stool collected | Enter the number (#) of months that the child has been followed and in which this monthly stool sample was collected for this particular child. |
| 08 | Recollection necessary? | Indicate if recollection is necessary. 2 grams are required for the 60m stool. |
| 09 | Fieldworker ID | Enter the fieldworker’s unique ID number who picked up the stool sample and/or visited the home. |

**SRF—Receiving and Storage of Stool Samples SOP**

1. **Purpose**

This document describes the procedures for receiving 60 stool sample collected from study subjects, and the assignment of Sample ID to the stool samples. The procedures are designed to maintain the integrity of clinical specimens by proper handling and storage and the management of associated records. Personnel handling the samples and data are trained to receive and handle clinical specimens and records.

1. **Materials**

Refrigerator

-70°C freezer

-20°C freezer

Wooden spatula

Sterile cryovials

2 ml screw cap tube that is compatible with the bead beater

Cryovial labels

Cryovial racks

Storage boxes

Scale

Stool receiving form (SRF)

1. **Methods**

**3.1Safety:**

1. Universal precautions must be followed while handling samples (<http://www.cdc.gov/ncidod/dhqp/gl_isolation_standard.html>).
2. Technicians must wear appropriate proper personal protective equipment (PPE) (e.g. gloves, masks, etc.) when handling samples as well as when handling ultra cold freezer items.
3. Disinfect work areas before and after performing these procedures.
4. Avoid using techniques that are likely to generate aerosols. Additional information can be found at <http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>.

**3.2 Transfer of samples to the laboratory:**

Proper transfer of samples from field to laboratory will require coordination between field workers and laboratory technicians. Collection and transport of samples should be carried out in clean, labeled, dry, leak-proof containers. Transport the samples to the laboratory as soon as possible after collection. Samples should be transported in an ice chest or insulated carrier containing adequate amount of cold packs as well as cushioning material to protect the samples. Do not use wet ice for transport of clinical specimens.

**3.3 Receipt of specimens:**

When the stool sample is received at the laboratory, the Sample ID is assigned using the Sample ID logbook provided to each individual site. After the Sample ID has been successfully assigned, the Sample ID is written on the existing label on all sets of stool samples made from this stool collection. All stool sample sets collected at that specific time will have the SAME Sample ID for each individual study subject. Any future aliquots of this stool sample collected at that specific time will also have the same Sample ID. Future stool samples collected a different time from the same individual will have different Sample IDs. Recollection samples have a different Sample ID than the initial sample’s Sample ID.

**3.4 Procedure for Aliquoting and Storage:**

1. REFER for MDF SOP: Microscopy Ova and parasite microscopy. The Lab will perform a direct prep on fresh stool and preserve stool in formalin for concentration
2. ARCHIVE for nucleic acid extraction and TAC: Many sites have found it easiest to weigh the aliquot that will be used for nucleic acid extraction when it is fresh. Weigh 180–220 mg stool or 200ul if liquid, into a 2 ml screw cap tube that is compatible with the bead beater. If less than 180mg is used, document the quantity, Freeze at -70 to -80 C until nucleic acid extraction.
3. FREEZE Aliquots: Freeze a quickly as possible and document time in to freezer. Refer to SOP “Barcode Sample Tracking” instructions for assistance in creating label for archiving/storage. Prepare a barcode label for each aliquot
4. ARCHIVE ADDITIONAL ALIQUOTS:  Suggest 4 vials .Store aliquots at -70 to -80˚C.

**4.0 Reporting:**

Record information on Stool Receiving Form (SRF). Please see the table below for assistance.

|  |  |  |
| --- | --- | --- |
| # | Question | Guidance |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| 01 | Sample ID | Write the 9-digit unique Sample ID. |
| 02 | Date of collection | Enter the date of collection (DD/MMM/YY) of the stool sample. Information located on SFC, question #1. |
| 03 | Time stool specimen was produced | Enter the time that the stool specimen was produced. If this time is not available, estimate if time cannot be confirmed by care giver. Information located on SFC, question #4. Enter NA if Cary Blair is not used (ie 60m collection) |
| 04 | Time stool specimen was picked up by Study Researcher / Nurse / Fieldworker | Enter the time that the stool specimen was picked up by the Study Researcher / Nurse / Fieldworker. Information located on SFC, question #2, time of visit. |
| 05 | Time stool specimen was preserved in Cary Blair | Enter the time that the stool specimen was preserved in Cary Blair. Information located on SFC, question #5. |
| 06 | Time stool specimen was received at field site/ laboratory | Enter the time that the stool specimen was received at the main laboratory or the intermediate field site, if appropriate. |
| 07 | Technician code | Enter the technician’s unique ID number who processed the specimen. |
| 08 | Unpreserved specimen? | Indicate whether or not an unpreserved specimen was received from the field. |
| 09 | Type of specimen | Indicate whether the stool specimen was a monthly, diarrheal, monthly recollection, and diarrheal recollection. Information located on SFC, question #6 and on sample container. Only M1 stools should be collected for 60m. |
| 10 | Month of stool collected | Enter the number (#) of months that the child has been followed and in which this monthly stool sample was collected for this particular child. For example, if a child has been followed for 14 months and the stool is being collected for his 14th monthly stool sample, enter 14. If the stool sample is a diarrheal stool sample, enter NA. |
| 11 | If recollection, what is Sample ID of initial sample? | If the stool sample is a recollection sample, enter the Sample ID of the initial sample. If the stool sample is not a recollection sample, enter NA. **Recollection samples have a different Sample ID than the initial sample’s Sample ID.** All sample IDs are unique values. |
| 12 | Amount of stool | Enter the number of grams of stool received. |
| 13 | Recollection? | Indicate if recollection was requested, and what type of recollection (monthly vs. diarrheal) is requested. Information located on SFC, question #8. 2 grams are required for the 60m stool. |
| 14 | Consistency | Indicate the consistency of the stool sample, WATERY (very loose, watery stool with very little solid material) = 01, LIQUID (loose stool taking the shape of the container) = 02, SOFT (semi-solid, smooth stool) = 03, FORMED (solid, firm stool) = 04 |
| 15 | Bloody? | Indicate if the stool specimen was bloody or not bloody. **If bloody, refer the child for medical treatment of suspected dysentery.** |
| 16 | Mucus? | Indicate if the stool specimen had mucus or did not have mucus. |
| 17 | Was Cary Blair used in the field? | Indicate yes or no if Cary Blair was used in the field. If yes, go to question #19. If no, go to question #18. |
| 18 | Time Cary Blair used in the laboratory | Indicate what time Cary Blair was used in the laboratory. |
| 19 | Was formalin used in the field? | Indicate yes or no if formalin was used in the field. If yes, go to question #21. If no, go to question #20. |
| 20 | Time formalin used in the laboratory. | Indicate what time formalin was used in the laboratory. |
| 21 | Time stool left field site laboratory | For SELECT SITES ONLY, indicate what time the stool sample left field site laboratory. If your site does NOT have an intermediate field site processing laboratory, please respond NA. |
| 22 | Time stool received at main testing laboratory | For SELECT SITES ONLY, indicate what time the stool sample was received at the main testing laboratory. If your site does not have an intermediate field site processing laboratory, please respond NA. |
| 23 | QNS set up? | Enter NA for the 60m stool. |
| 24 | Time specimen placed in  -80 freezer | **Enter the time vials were placed into the -70 to -80**˚C **freezer** |

**MDF—Microscopy for the Detection of Ova and Parasites in Stool**

1. **Purpose**

To detect ova and parasites in stool specimens by the wet prep microscopic examination of Direct fresh stool and formalin-concentration preserved stool concentrate. Parasitic organisms can be detected in preparations made from stool samples using light microscopy. The organisms are identified morphologically by examining the shape, size, nuclear and cytoplasmic characteristics of the eggs, cysts, and parasites (trophozoites or worms).

1. **Quality control:**
   1. Atlases, photographs and charts of parasites will be available and used by laboratory microscopists for the confirmation of positives.
   2. Digital photographs may be taken of any questionable results and can be reviewed internally or sent to the Desktop for confirmation or resolution.
   3. **External quality assurance:** Five formalin stool concentrates for wet prep microscopy will be sent to site laboratories every 6 months Results will be compared to peer labs. A pass rate of 80% must be maintained.
   4. **Internal quality assurance:** At least annually, the bench technologist will re-examine five stored, randomly selected formalin-fixed samples that have the original results blinded. The results of the re-examination will be compared to the original result.

**3.0 Safety:**

**3.1** Specimens should be handled, processed and disposed of using standard guidelines for biohazardous materials.

* 1. Spills should be immediately disinfected with 6% hydrogen peroxide (preferred) or 10% bleach.
  2. Lab bench should be disinfected at the completion of every work day

**4.0 Specimen**:

Refer to the Stool Specimen Collection and Transport SOP for details. Fresh and Formalin preserved stools are examined.

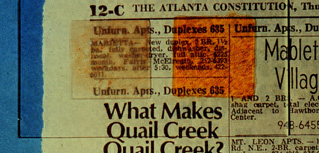
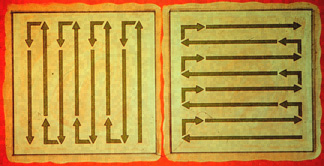
* 1. Fecal specimens : 2 grams are collected into sterile empty containers with no additives.
  2. A portion ( ~1 gram) of each specimen is formalin preserved in the field or in the lab. Use 10% formalin at a ratio of one part stool to three parts formalin preservative
  3. Stool ova and parasites in formalin are preserved and may be tested in batches.
  4. The specimens should not be frozen or placed in an incubator because of rapid parasitic degradation.

**5.0 Wet Mount Preparation:** Protozoan trophozoites, cysts, oocysts, and helminth eggs and larvae may be seen and identified using a wet mount identification technique. A wet prep is performed both directly from fresh stool and also from the sediment after concentration

**5.1** Direct Prep from fresh stool: If the stool specimen is still somewhat solid, add a drop or two of saline to the specimen and mix to obtain the correct thickness

**5.2** Wet prep from Concentration Sediment: After concentration prepare a wet prep a drop or two of saline may need to be added to obtain the optimal density for microscopic exam.

**5.3**  Ideally, two smears can be prepared on one slide, of which one can be stained with iodine. Thickness of the wet mount should be sufficiently thin to read print through it as figure A illustrates. Systematically scan the entire coverslip area using the 10× objective as illustrated in  **figure B.** If something suspicious is seen, a higher magnification may be necessary for identification Use 40x to identify further.

**Figure A**   **Figure B** 

**6.0 Microscopic Examination :** Protozoan cysts and helminth eggs and larvae may be seen and identified.

**6.1** Examine the slides using a microscope at 10X and 40X:

a. Systematically scan the entire coverslip with the 10X objective (low power). If something suspicious is seen, turn to the 40X objective for more detailed exam.

* 1. Scan at least one-third of the coverslip with the 40X objective (high dry power), even if nothing suspicious has been seen at 10X.
  2. Consult photographs and refer to parasitology atlas for identification guidance. Study site may email problem or unknown parasite photographs to the microbiology subcommittee for consultation.

**Fecal Parasite Concentration Using the Evergreen FPC**

**1.0 Purpose**

The concentration method is designed to separate protozoan cysts, oocysts and helminth eggs and larvae from fecal debris by centrifugation and differences in specific gravity. The suspension fluid (formalin and ethyl acetate) is less dense than cysts and eggs and thus they are found in the sediment in the bottom of the tube. Straining and defatting steps (using ethyl acetate to dissolve fecal fats and make the fecal debris float) remove fecal debris and clarify the specimen.

1. **Specimen:** Stool preserved in 10% formalin at a ratio of one part stool to three parts formalin preservative. Formalin preserved stools may be batch tested.
2. **Materials**

FPC Fecal Parasite Concentration kit (Evergreen Scientific, (800)421-6261)

10% buffered (or unbuffered) Formalin: Combine and store in a tightly closed bottle (pH ~7.4):

100 ml formaldehyde (37% solution) + 900 ml water

For buffered, add 12g Na2HPO4 + 3g KH2PO4

Lugol’s Iodine:

Dissolve 10 g potassium iodide (KI) + 5 g powdered iodine crystals in 100 ml distilled water in a brown bottle (excess iodine crystals should remain on the bottom of the bottle). Store at room temperature for up to 1 year.

Working solution = dilute 1:5 with distilled water (prepare fresh every 10-14 days)

0.85% NaCl

Ethyl acetate

Centrifuge

Light microscope with 10x and 40x objectives

Disposable transfer pipets

Microscope slide & coverslip

**Quality Control:**

1. Check reagents daily, formalin should appear clear, without contamination.
2. Known positive control specimens should be examined quarterly.
3. Microscope should be cleaned and have the ocular micrometer calibrated annually.
4. Centrifuge speed calibration should be performed annually.
5. **Methods:**

Concentration Procedure for manual use a commercial concentration kit , Evergreen Fecal Parasite Concentrator (FPC) kit.

**Evergreen Fecal Parasite Concentrator (FPC) kit:** as per product insert

1. Line up the flat-bottom tubes in a rack; label tubes with laboratory accession number.

2. Place the conical centrifuge tubes (with the green filtration screen attached) in a rack directly behind the flat-bottom tubes.  Label tubes with laboratory accession number.

3. Add the following to the flat-bottom tube in the order specified:

A. 9 ml of 10% formalin

B. Two spoonfuls of formalinized feces (1.0 g)

C. Three drops of Triton X-100

D. 3 ml of ethyl acetate

4. Attach the conical centrifuge tube (with strainer attached with the aerator tube extended 3.5 cm above the top of the green mid-piece screen so that it extends above the fluid level when the tube is inverted.) to each flat bottom tube.

BE SURE TO MATCH THE CORRECT TUBE NUMBERS WITH EACH OTHER.  Tighten each connection to prevent leakage.

5. Shake vigorously for 30 seconds.

6. Flip the tubes over and allow the mixture to filter into the strainer into the conical tube.  If the sample stops flowing, tap the conical tube on the countertop to re-start the transfer.

7. Unscrew the centrifuge tube/strainer assembly and discard.

8. Screw a white plastic cap onto each conical centrifuge tube.

9. Centrifuge conical tubes at 500 x g for 10 minute. Ring the fecal plug using an applicator stick and decant all of the supernatant.

10. While holding tube upside down, remove fluid from walls of the tube with a cotton-tipped swab.

11. Add 1-2 drops of saline to resuspend the sediment and place a drop on a slide and cover with a 22x22 cover slip, examine microscopically

**Reporting:**

1. Record results on Microscopy Data Form (MDF). See below for additional guidance.

|  |  |  |
| --- | --- | --- |
| # | Question | Guidance |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| 01 | Sample ID | Write the 9-digit Sample ID. |
| 02 | Date specimen was received | Enter the date that the specimen was received. |
| 03 | Technician code | Enter the technician’s unique ID number who processed the sample. |
| 04 | Was wet prep microscopy performed? | Indicate whether or not wet prep microscopy was performed. If no, STOP completing the form. |
| 05 | Were parasites observed? | Indicate yes if parasites were observed and no if parasites were not observed upon wet prep microscopy. If no, continue to question #42. If yes, continue to question #6. |
| 06-39 | Which parasite was observed? | For each parasite listed, indicate whether or not this parasite was observed using either concentrate wet prep and/or direct wet prep. |
| 40-41 | Other parasites observed? | Indicate whether additional parasites not listed were observed using either concentrate wet prep and/or direct wet prep. If additional parasites were observed, complete parasite reporting log. |
| 42 | Technician code | Enter the technician’s unique ID number who performed the wet preps. |
| 43 | Date specimen was tested for parasites | Enter the date that the specimen was tested for parasites. |
| 44 | Was a modified acid-fast stain performed? | Indicate whether or not a modified acid-fast stain was performed. If the stain was not performed, STOP; form is complete. The modified acid-fast stain is not part of the protocol for the 60m sample. |
| 45 | Did modified acid-fast stain detect an organism? | Indicate whether or not an organism was detected and what organism was detected. |
| 46 | Technician code | Enter the technician’s unique ID number who performed the modified acid-fast stain. |
| 47 | Date | Enter the date (DD/MMM/YY) that the modified acid-fast stain was performed. |

**QIAamp Fast DNA Stool Mini Kit SOP**

**Purpose**

This procedure describes a modified extraction procedure to isolate both DNA and RNA from fecal specimens using the QIAmp Fast DNA Stool MiniKit. The fecal sample undergoes a lysate preparation process and includes mechanical disruption (bead beating), removal of inhibitors, purification and elution of DNA and RNA using spin columns. Extrinsic controls PhHV (Phocine Herpesvirus) and MS2 are added to each sample during the lysate preparation to evaluate extraction and amplification efficiency. The extracted total nucleic acid (TNA) is then stored at -80°C for testing. To rule out contamination during the extraction process, a blank is also processed through the complete protocol each day extractions are performed.

**Important Notes to Maximize Downstream Assay Performance**

1. PhHV and MS2: Careful preparation, storage and use of the MS2 and PhHV are essential because detection of these extrinsic controls is necessary to accurately evaluate the pathogen target PCR results. Detection of MS2 in the sample confirms the extraction and amplification of RNA and DNA were successful. When MS2 is not detected in the sample, it is not possible to accept the results of other RNA targets that were not detected, thus negative RNA target results in that sample would be invalid. MS2 and PhHV detection rates of <90% or CTs consistently above 32 for either PhHV or MS2 may indicate a procedural or reagent problem and should be investigated before continuing with extractions.
2. Extraction Blank: Processing a blank each day/batch of extractions helps to validate sample results in downstream assays. The extraction blank should be positive only for MS2 and PhHV. A pathogen target detected in an extraction blank may indicate a wider contamination problem. Positive results for that target in any sample that was extracted on that same day or within that batch would be invalid. The lab should address possible sources of the contamination before continuing with extractions. The sensitivity of PCR assays requires adherence to good laboratory practices to avoid cross-contamination between samples and laboratory contamination.

**A. Materials**

1. QIAamp Fast DNA Stool Kit (cat 51604, 50 preps) plus additional collection tubes (~2 per sample, Qiagen, Cat 19201)
2. PhHV: store at 4°C
3. MS2 working concentration (see Appendix 1 for instruction)
4. Bead beater
5. Vortexer
6. Microcentrifuge
7. Water bath or dry bath 70°C
8. Dry baths 95°C
9. -70 to -80°C freezer
10. Pipettes and Pipet tips
11. 96%-100% Ethanol
12. 2ml screw cap tubes, compatible with bead beater
13. 2.0 ml microcentrifuge tubes
14. Glass beads, acid-washed 212-300 µM (50-70 U.S. sieve) (Sigma G1277-500G)
15. 0.5 ml screw cap tubes for TNA storage

**B. QIAamp Fast DNA Stool Mini Kit Reagent Preparation and Kit Components**

Each kit can do 50 sample extractions

Store all kit components at Room Temp (15-25°C) for up to 1 year after opening.

1. InhibitEX Buffer: Mix Buffer thoroughly by shaking before use. If a precipitate has formed, incubate at 35°C to 70°C for ~15 minutes to ensure any precipitate has fully dissolved. A color change to orange during storage can be expected. It does not effect the buffer performance.
2. Buffer AL: Mix Buffer AL thoroughly by shaking before use. If a precipitate has formed, incubate at 35°C to 70°C for ~15 minutes prior to use to ensure all precipitate has fully dissolved.
3. ProteniaseK: If ambient temperature often exceeds 25°C, store at 4-8°C for up to one year. Never add proteinase K directly to Buffer AL.
4. Buffer AW1: Buffer AW1 is supplied as a concentrate. Before using for the first time, add 25 ml ethanol (96–100%) as indicated on the bottle. Thoroughly mix before use.
5. Buffer AW2: Buffer AW2 is supplied as a concentrate. Before using for the first time, add 30 ml ethanol (96–100%) to Buffer AW2 concentrate as indicated on the bottle. Thoroughly mix before use.
6. Buffer ATE

**C. Bead Beating Time: instrument adjustments**

1. Bead disruption for too long will overheat the sample and cause nucleic acid degradation, too little time is ineffective and will not enhance the extraction of DNA. Care should be taken in determining bead beating time for the instrument used. If immediately after bead beating the tube is hot, decrease the time.
2. Fastprep FP120 from Bio101 Thermo Electronic Corporation (Fisher NC9646109)- Bead disruption at maximum speed for 3 minutes is sufficient without over heating the preparation.
3. BioSpec Mini-Beadbeater (BioSpec 693, Mini-Beadbeater-8) -Bead disruption at maximum speed for 2 minutes is sufficient.

**D. Procedure Notes:**

1. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.
2. Change pipet tips between all liquid transfers. The use of aerosol-barrier pipet tips is recommended.
3. After all vortexing steps, to avoid contamination pulse centrifuge the tubes to remove drops from the inside of the lid.
4. Pipet the sample into the QIAamp Mini spin column without moistening the rim of the column.
5. Avoid touching the QIAamp membrane with the pipet tip.
6. Close the QIAamp Mini spin column before placing it in the microcentrifuge.
7. Open only one QIAamp Mini spin column at a time.
8. For efficient parallel processing of multiple samples, fill a rack with collection tubes

**E. Specimens :**

**Use screw cap tubes compatible with the bead beater**

1. Fecal Sample: Fresh (refrigerated up to 18 hours) or frozen long term at -70°C

Weigh 180–220 mg stool or 200ul if liquid, into a 2 ml screw cap tube that is compatible with the bead beater. If less then 180mg is used, document the quantity, but there is no need to reduce the amounts of buffers when using smaller amounts of sample.

2. Blank (no fecal specimen) include once each day extractions are performed:

Begin with step 3 below and process through the entire protocol

**F. Procedure**

1. Mix InhibitEX Buffer thoroughly by shaking before use. If a precipitate has formed, Incubate at 37-70°C for ~15 minutes or until all precipitate has fully dissolved
2. Prepare fresh each day: Add PhHV and MS2 to InhibitEX buffer: prepare only enough for N+1 samples to be processed that day: 1ml InhibitEX buffer +1ul PhHV+ 1ul MS2 is needed per sample (e.g. For 10 samples 11ml InhibitEX+ 11ul PhHV+11 ul MS2), mix well.
3. Add : ~370 mg (one eppendorf tube capful) of Sigma beads to the aliquot of stool (or blank tube)
4. Add 1 ml of the InhibitEX/PhHV/MS2 solution into each fecal specimen tube (or blank).
5. Vortex for 1 min
6. Bead beat at maximum speed for 2-3 minutes-(instrument dependent, see section C)
7. Incubate the suspension for 5 min at 95°C.
8. Vortex for 15 s then centrifuge sample at full speed ( approximately 20,000g) for 1 min to pellet the stool particles.
9. Pipet 25 μl proteinase K into a new 2 ml microcentrifuge tube, (snap caps acceptable).
10. Pipet 600 μl supernatant from step 8 into the 2 ml microcentrifuge tube containing proteinase K. Note: Do not transfer any solid material, if necessary centrifuge sample again.
11. Add 600 μl Buffer AL. Note: Do not add proteinase K directly to Buffer AL.
12. Vortex for 15 s, Mix thoroughly to form a homogeneous solution.
13. Incubate at 70°C for 10 min.

Centrifuge briefly to remove drops from the inside of the tube lid.

1. Add 600 μl of ethanol (96–100%) to the lysate, and mix by vortexing.

Centrifuge briefly to remove drops from the inside of the tube lid.

1. Label QIAamp spin column lid and place in a 2 ml collection tube. Carefully apply 600 μl of the lysate from step 14 to the QIAamp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min.
2. Retain QIAamp spin column, place the column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.
3. Repeat 2 more times to use all of the lysate: carefully apply an additional 600 μl of the lysate from step 14 to the QIAamp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min. Retain the column. Discard the collection tube and filtrate. Repeat until all of the lysate ( ~1800 ul in total) has been through the spin column.
4. Place the spin column in a new collection tube.
5. Open the QIAamp spin column and add 500 μl Buffer AW1. Close the cap

and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml

collection tube, and discard the collection tube containing the filtrate.

1. Open the QIAamp spin column and add 500 μl Buffer AW2. Close the cap

and centrifuge at full speed for 3 min. Retain QIAamp spin column, discard the collection tube containing the filtrate.

1. To eliminate the chance of possible Buffer AW2 carryover place the QIAamp spin column in a new 2 ml collection tube and discard the old collection tube with filtrate. Centrifuge at full speed for 3 min. Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications.
2. Elute the total nucleic acid: Transfer the QIAamp spin column into a labeled microcentrifuge tube. Open the QIAamp spin column and pipet 200 μl Buffer ATE directly onto the QIAamp membrane. Close the cap and incubate for 1-3 min at room temperature, then centrifuge at full speed for 1 min to elute the TNA .
3. **Save the filtrate**: this is the TNA. Discard the column.
4. Prepare two tubes for aliquot storage. Pipet 100 ul of the extracted TNA into each tube. Store at -70 to –80°C.

**Appendix 1 Preparation and Storage of MS2 Phage**

The phage MS2 is incorporated during extraction as an extrinsic control in order to evaluate the extraction and amplification efficiency of RNA and DNA. This protocol describes the preparation and storage of MS2.

Material

1. Lyophilized MS2 phage (ATCC, cat# 15597-B1)

2. Sterile PBS

3. 0.5 ml cryovials

1. Prepare Concentrated Stock Aliquots

a. Reconstitute the MS2 pellet in 2 ml of sterile PBS, mix thoroughly until no clumps are visible. The solution is turbid.

b. Make 25 µl aliquots and store at -80C. This is the “Concentrated Stock”. Use each aliquot only once. Do not freeze- thaw.

Concentrated stock stored at -80 and never thawed is stable for at least 1 year.

2. Prepare Working Solution Aliquots

a. Dilute “Concentrated Stock” 50 times: Add 20 µl concentrated stock to 980 µl of PBS, mix thoroughly. This is the working solution.

b. Make 50 µl aliquots and store at -80C. Aliquots stored at -80 and never thawed are stable for at least 1 year.

c. Once thawed, do not refreeze working solution or store any unused in the refrigerator.

3. TNA Extraction:

Use 1 µl working solution per sample for TNA extraction (refer to extraction SOPs). Discard any remaining MS2 working solution each day, i.e. do not use any refreeze- thawed working solution. Always prepare the lysate buffer/MS2/PhHV mix fresh each day.

**SES & Food Security**

**FSE—Followup SES Questionnaire**

**I. Purpose**

The Followup Socioeconomic Status Form (FSE) will be administered in each MAL-ED household at 60 months.

**II. Material**

Followup Socioeconomic Status Form (FSE), pen and clipboard.

**III. Methods**

The following people should respond to the questions on this form: head of household, mother of the child, and primary caregiver (if different from the mother).

* **Head of household** is defined as the person who contributes most financially to the household.
* **Mother** is defined as the biological mother of the child, or the woman who gave birth to the child, not someone acting as a caregiver who may consider herself the child’s mother.
* **Primary caregiver** is the person who most often cares for the child, including taking care of the child when he/she is sick, feeding the child, etc.

We recognize that some of the questions on this form are redundant (#2, 3, 5, 6, and others) as we’ve asked these same questions during the birth to 24 month period. In order to keep the form consistent with the previously used form, we ask that you record answers for these questions once again (this will also allow us a double check on the data). The first 8 questions should be answered by the head of household, if this person is different from the child’s mother or primary caregiver. Questions 9-20 are for the mother of the child. If the mother is deceased skip to question 21. If the mother is unavailable, return to the household at another time to complete these questions. If the mother is alive, it is important that she respond to questions 9-20, even if she is not the child’s primary caregiver. Questions 21-28 should be addressed to the child’s primary caregiver. *Complete these questions only if the mother is not the primary caregiver.*  If the mother is the primary caretaker skip to question 29.The remaining questions should be answered by the child’s primary caregiver. Questions 29-88 should be answered by the child’s primary caregiver.

**Header information**

The first section, including date, fieldworker ID and child ID should be completed by the fieldworker once the mother has consented to participate.

|  |  |  |
| --- | --- | --- |
| **#** | **Question** | **Guidance** |
| **DEMOGRAPHIC QUESTIONS** | | |
| *Questions for head of household (if mother/primary caregiver of child is the head of household skip to question 9)* | | |
| 1 | What is your age? | This is the age of the head of household in years. If the head of household does not know her or his exact age ask for an estimate, and enter that number. |
| 2 | *(Record sex)* | Record the sex of the head of household. You can answer this question using observation rather than asking it. |
| 3 | What is your relationship to [CHILD’S NAME]? | This is the head of household’s relationship to the infant/child. Refer to the infant/child by name when asking this question. |
| 4 | Are you currently married, divorced, widowed, or never married?  *If never married, skip to question 6.* | For this question, married is defined as either legally married or cohabiting with a partner. Similarly, divorced includes legally divorced or no longer cohabiting with a partner. |
| 5 | How old were you when you got married for the first time? | This question asks the age of the head of household when she or he was first married. Again, marriage includes cohabiting with a partner as well as legal marriage. |
| 6 | Have you ever attended school?  *If no, skip to question 9.* | This question refers to formal school only, and includes any formal schooling beginning with kindergarten. |
| 7 | How many years of schooling have you completed? | This question is asking about years of schooling completed, beginning with kindergarten and through any university or post-university education. If the respondent began a year of school but did not complete it, do not include that year. If the respondent repeated any years of schooling, only include that grade level once. For example, if the respondent repeated 5th grade, include that as 1 year only, rather than 2 years. |
| 8 | *If younger than 25 years old:* Are you currently attending school or college? | This question refers to formal school only, but might include technical schools as well as university programs. |
| *Questions 9-20 are for the mother of the child. If the mother is deceased skip to question 21. If the mother is unavailable, return to the household at another time to complete these questions. Complete these questions even if the mother is not the primary caregiver for the enrolled child.* | | |
| 9 | What is your age? | This is the age of the child’s mother. If she does not know her exact age ask for an estimate, and enter that number. |
| 10 | Are you currently married, divorced, widowed, or never married?  *If never married, skip to question 12.* | For this question married is defined as either legally married or cohabiting with a partner. Similarly, divorced includes legally divorced or no longer cohabiting with a partner. |
| 11 | How old were you when you got married for the first time? | This question asks the age of the mother when she was first married. Marriage includes cohabiting with a partner as well as legal marriage. |
| 12 | Have you ever attended school?  *If no, skip to question 15.* | This question refers to formal school only, and includes any formal schooling beginning with kindergarten. |
| 13 | How many years of schooling have you completed? | This question is asking about years of schooling completed, beginning with kindergarten and through any university or post-university education. If the respondent began a year of school but did not complete it, do not include that year. If the respondent repeated any years of schooling, only include that grade level once. For example, if the respondent repeated 5th grade, include that as 1 year only, rather than 2 years. |
| 14 | *If younger than 25 years old:* Are you currently attending school or college? | This question refers to formal school only, but might include technical schools as well as university programs. |
| 15 | How old were you when you first became pregnant? | This question asks about the mother’s age when she became pregnant for the first time. In this case, include all pregnancies, whether or not they resulted in live births. |
| 16 | How many pregnancies have you had in your lifetime? | This question asks about the number of pregnancies a woman has had in her lifetime. Include all pregnancies, whether or not they resulted in live births. |
| 17 | How many live births have you had in your lifetime? | This question asks about the number of live births a woman has had in her lifetime. Include only live births. This number should be less than or equal to the number of pregnancies. |
| 18 | Are all of these children still alive?  *If yes, skip to question 20.* | This question asks about the number of children who are still alive, out of the number of live births. This number should be less than equal to the number of live births. |
| 19 | How many children have died? | This question asks about the number of children, out of the live births, who have died. For many women the answer might be 0. Otherwise this number should be less than or equal to the number of live births from question 17. |
| 20 | Are you the primary caregiver for [CHILD’S NAME]? | The primary caregiver is the person who most often cares for the child, including taking care of the child when he/she is sick, feeding the child, etc. If the answer is no, ask questions 21-28 to the child’s primary caregiver. Is the answer is yes, skip questions 21-28 and continue with question 29. |
| *Questions 21-28 should be addressed to the child’s primary caregiver. Complete these questions only if the mother is not the primary caregiver. If the mother is the primary caregiver skip to question 29.* | | |
| 21 | What is your relationship to [CHILD’S NAME]? | This is the primary caregiver’s relationship to the infant/child. Refer to the infant/child by name when asking this question. |
| 22 | How old are you? | This is the age of the child’s primary caregiver. If he or she does not know his/her exact age ask for an estimate, and enter that number. |
| 23 | Are you currently married, divorced, widowed, or never married?  *If never married, skip to question 25.* | For this question married is defined as either legally married or cohabiting with a partner. Similarly, divorced includes legally divorced or no longer cohabiting with a partner. |
| 24 | How old were you when you got married for the first time? | This question asks the age of the primary caregiver when she/he was first married. Marriage includes cohabiting with a partner as well as legal marriage. |
| 25 | What is your relationship to [NAME OF HEAD OF HOUSEHOLD]? | This question asks about the relationship of the caregiver to the head of household. |
| 26 | Have you ever attended school?  *If no, skip to question 29.* | This question refers to formal school only, and includes any formal schooling beginning with kindergarten. |
| 27 | How many years of schooling have you completed? | This question is asking about years of schooling completed, beginning with kindergarten and through any university or post-university education. If the respondent began a year of school but did not complete it, do not include that year. If the respondent repeated any years of schooling, only include that grade level once. For example, if the respondent repeated 5th grade, include that as 1 year only, rather than 2 years. |
| 28 | *If younger than 25 years old:* Are you currently attending school or college? | This question refers to formal school only, but might include technical schools as well as university programs. |
| **SOCIO-ECONOMIC STATUS QUESTIONS** | | |
| 29 | How long has your family lived in this house? | This question asks about the length of time that the child’s family has lived in their current home. If the head of household’s family lived in the home prior to the child’s mother living there, record the length of time that the head of households family has lived in the home. The goal is to understand how long the extended family of the child and lived in this home, not the child or mother specifically. |
| 30 | How many rooms are there in your house? | The total number of rooms in the house includes the veranda, the living and sleeping rooms, as well as attached kitchens used only by this household. It excludes storerooms and separate kitchens that are shared with other families/households. |
| 31 | How many rooms in this household are used for sleeping? | This question is seeking information on which rooms in the house are usually used for people to sleep. For example, this would not include the kitchen or toilet facilities. |
| 32 | How many people usually sleep in this household? | This question asks about the number of people who usually sleep in this household on an average night. |
| 33 | Does your household pay any domestic workers? | Domestic workers are defined as non-family members who work in the household for pay. |
| 34 | What is the main source of drinking water for members of your household?  *If answered 04, 05, 06, 07, or 08 skip to question 37.* | The purpose of this question is to assess the cleanliness of the household drinking water by asking about the household’s main source of water. If drinking water is obtained from several sources, probe to determine the source from which the household obtains the majority of its drinking water. If the source varies by season, record the main source used at the time of interview. Below are explanations of each of the response categories.  **01= Piped into dwelling:** Pipe connected with in-house plumbing to one or more taps, e.g. in the kitchen and bathroom. Sometimes called a house connection.  **02= Piped to yard/plot:** Pipe connected to a tap outside the house in the yard or plot. Sometimes called a yard connection.  **03= Public tap or standpipe:** Public water point from which community members may collect water. A standpipe may also be known as a public fountain or public tap. Public standpipes can have one or more taps and are typically made of brickwork, masonry or concrete.  **04= Tubewell or borehole:** A deep hole that has been driven, bored or drilled with the purpose of reaching ground water supplies. Water is delivered from a tubewell or borehole through a pump which may be human, animal, wind, electric, diesel or  solar-powered.  **05= Protected well:** A well that is (1) protected from runoff water through a well lining or casing that is raised above ground level and a platform that diverts spilled water away from the well and (2) covered so that bird droppings and animals cannot fall down the hole. Both conditions must be observed for a dug well to be considered as protected.  **06= Unprotected well:** A well which is (1) unprotected from runoff water; (2) unprotected from bird droppings and animals; or (3) both.  **07= Surface water:** Water located above ground and includes rivers, dams, lakes, ponds, streams, canals, and irrigation channels  **08= Other** |
| 35 | Is your piped water supply continuous or is it sometimes interrupted? | This question is for subjects who responded either “piped into dwelling”, “piped to yard/plot”, or “public tap or standpipe” to question 34. If continuous, skip to question 37. |
| 36 | How often do these interruptions last? | If the subject responded that their water is sometimes interrupted to question 35, ask how often these interruptions usually last for. If the interruptions are seasonal, enter “05” for “more than 24 hours”. |
| 37 | What is the main source of water used by your household for other purposes such as cooking and hand-washing? | This question asks about the main source of water used for purposes other than drinking. In many cases it will be the same response as that given to question 34. |
| 38 | Do you pay or barter for water? | This question asks about whether the household ever pays or barters for any source of water, either formally or informally. |
| 39 | Where is the water source located? | This question asks about the location of the household’s drinking water source. |
| 40 | How long does it take to go there, get water and come back in one trip? *If water is located on the premises, response is 000.* | If the source of drinking is located within the dwelling or yard/plot, or if the household relies on rainwater, enter “000” as the response to this question. Otherwise, include the time it takes to get to the source, wait to get water (if necessary), and get back to the house. Record the time it takes to get water by whatever means of transportation the person generally uses, whether the person walks or rides a bicycle or motor vehicle. Convert answers given in hours to minutes. Put zeroes in front of the response if necessary; for example, “30 minutes” would be ‘030,’ and “one hour and a half” would be ‘090.’ |
| 41 | Who is the main person in the household who goes to fetch water from this source? | The purpose if this question is to know which family member usually performs the task of fetching water. Knowing which member of the household usually hauls the water gives us an idea of whether gender and generational disparities exist with respect to water hauling responsibilities. If the respondent answers that there are several members from the household who perform this chore, emphasize that you are interested in the person who usually fetches the water (i.e., most of the time). |
| 42 | Do you treat your water in any way to make it safer to drink? | The purpose of questions 42 and 43 is to know whether the household drinking water is treated within the household and if so, what type of treatment is used. The type of treatment used at the household level provides an indication of the safety of the drinking water used in the household. |
| 43 | What do you usually do to the water to make it safer to drink?  *Note that the order of response categories has changed since the PSE for this question.* | The meaning of response categories for this question are:  **01= Let it stand and settle:** Holding or storing water undisturbed and without mixing long enough for larger particles to settle out or sediment by gravity  **02= Solar disinfection:** Exposing water, which is stored in buckets, containers, or vessels, to sunlight.  **03= Using a water filter (ceramic/sand/composite/etc.):** The water flows through a media to remove particles and at least some microbes from water. Media used in filtering systems usually include ceramic, sand and composite.  **04= Strain it through a cloth:** Pouring water through a cloth which acts as a filter for collecting particulates from the water  **05= Add bleach/chlorine:** Use of free chlorine to treat drinking water. Free chlorine may be in the form of liquid sodium hypochlorite, solid calcium hypochlorite, or bleaching powder  **06= Boil:** Boiling or heating of water with fuel  **07= Other** |
| 44 | Do you wash your hands after helping your child defecate? | Questions 44-46 ask about the respondent’s hand-washing practices. This question asks about whether the respondent washes her hands after helping her child defecate, which includes changing diapers. In all cases hand-washing must include use of soap. |
| 45 | Do you wash your hands before preparing food? | This question asks about whether the respondent washes her hands with soap directly before preparing food. |
| 46 | Do you wash your hands after using the toilet? | This question asks about whether the respondent washes her hands with soap after using the toilet herself. |
| 47 | Do you use toilet paper? | This question refers to whether the respondent uses toilet paper after urinating or defecating herself (as opposed to helping her child). |
| 48 | What kind of toilet facility do members of your household usually use?  *Note that the order of response categories has changed since the PSE for this question.* | The purpose of this question is to obtain a measure of the sanitation level of the household, since toilet facilities are important for disease control and health improvement. Below are some definitions for the terms used in the codes:  **01= No facility/bush/field or bucket toilet:** The household has no facility and/or uses the bush, field, or a bucket toilet near the household.  **02= Pit latrine without flush (with slab):** Excreta is deposited without flushing directly into a hole in the ground. Pit latrines without flush include:  - Ventilated improved pit latrine (VIP): A latrine ventilated by a pipe extending above the latrine roof. The open end of the vent pipe is covered with gauze mesh or fly-proof netting and the inside of the superstructure is kept dark.  - Pit latrine with slab: A latrine with a squatting slab, platform or seat firmly supported on all sides which is raised above the surrounding ground level to prevent surface water from entering the pit and for ease of cleaning.  **03= Flush** **to piped sewer system:** A system of sewer pipes (also called sewerage), that is designed to collect human excreta (feces and urine) and wastewater and remove them from the household environment. Sewerage systems consist of facilities for collection, pumping, treating and disposing of human excreta and wastewater.  **04= Flush** **to septic tank:** An excreta collection device consisting of a watertight settling tank normally located underground, away from the house or toilet.  **05= Flush** **to pit latrine**: A system that flushes excreta to a hole in the ground.  **06= Flush** **to somewhere else** A system in which the excreta is deposited in or nearby the household environment in a location other than a sewer, septic tank, or pit, e.g., excreta  may be flushed to the street, yard/plot, drainage ditch or other location.  **07= Other**  **08=Pit Latrine without slab**  A latrine without a squatting slab, platform or seat.  An open pit is a rudimentary hole in the ground where excreta is collected.  **Additional Notes: This change was made to distinguish between WHO ‘improved’ vs ‘unimproved’ categories for WAMI score calculations. Also, the Spanish version of the FSE used in at PEL was translated differently so that option 02 is an ‘unimproved’ latrine whereas in other sites this is an ‘improved’ latrine.** |
| 49 | Do you share this toilet facility with other households? | Question 49 asks about whether the toilet facilities are shared with one or more other households. In question 50 we want to find out how many households use the same facility. This is an important measure of the level of hygiene in the household. |
| 50 | How many households use this toilet facility? | This question asks how many households regularly use the same toilet facility as the respondent household. If 10 or more households use this toilet facility enter “10”. |
| 51 | Does your household ever have electricity?  *If no, skip to question 55.* | This question asks whether the household ever has access to electricity. Even if the electricity access is irregular or unreliable, respond yes “01” to this question. |
| 52 | Is your electricity supply continuous year-round, or is it sometimes interrupted?  *If continuous, skip to question 55.* | This question asks whether the household’s electricity supply is ever interrupted. |
| 53 | *If sometimes interrupted:* How long do these interruptions usually last? | This question asks, when household electricity supply is interrupted, how long do these interruptions usually last. The respondent should give an approximate response that captures what happens most often when interruptions take place. |
| 54 | In case of discontinued power supply what source does this household usually use? | If there is no alternate electricity supply in cases of discontinued power, enter “NA”. |
| *The answers to these questions on ownership of certain items will be used to form a rough measure of the socioeconomic status of the household. Read out each item and enter the answer given for each item. Do not leave any item(s) blank. If the respondent reports that a household item such as a radio is broken, try to find out how long it has been broken and whether it will be fixed. If the item appears to be out of use only temporarily, enter ‘01’ for Yes. Otherwise, enter ‘00’ for No. If household assets were donated or given to the household, either as gifts or as part of a poverty alleviation program, they should still be included as long as they are in working form.* | | |
| 55 | Does your household have an iron (either charcoal or electric)? |  |
| 56 | Does your household have a mattress? |  |
| 57 | Does your household have a chair or bench? |  |
| 58 | Does your household have a sofa? |  |
| 59 | Does your household have a cupboard? | This includes cupboards with shutters and open shelves. |
| 60 | Does your household have a table? |  |
| 61 | Does your household have an electric fan? |  |
| 62 | Does your household have a radio or transistor? |  |
| 63 | Does your household have a computer? | This includes all computers that can be used for word processing and are in working form. |
| 64 | Does your household have a television? | This includes both black and white and color TVs. |
| 65 | Does your household have a mobile telephone? | This refers to whether any member of the household owns a working mobile phone. |
| 66 | Does your household have a refrigerator? |  |
| 67 | Does your household have a watch or clock? | This refers to any functioning watch or clock, regardless of quality. |
| 68 | Does your household have a bicycle? | A small child’s bicycle is primarily a toy and should not be recorded here. |
| 69 | Does any member of your household own a bank account? | This refers to formal bank accounts only, and does not include microfinance structures that operate outside of the formal banking system. |
| 70 | Does this household own any agricultural land?  *If no, skip to question 72.* | Ownership of agriculture land is another important indicator of the socioeconomic status of the household. First ask question 69 to find out whether any member of the household owns any land that can be used for agriculture. If the answer is YES, ask question 70 on the number of acres owned altogether by the members of the household. Record the answer in the respective boxes. |
| 71 | How much agricultural land does this household own? |  |
| 72 | Does your household own cows, bulls, or buffaloes? | For questions 72-73, answer “yes” if the household owns any of the animals listed, regardless of the number. |
| 73 | Does your household own chickens or ducks? |  |
| 74 | Do you ever heat your house?  *If no, skip to question 76.* |  |
| 75 | What is the primary source of fuel used for heating in your household? | If the household uses more than one fuel for heating, find out the fuel used most often and enter the code for that fuel. |
| 76 | What type of cooking stove is mainly used in your house? | In these questions we want to find out whether the food in the household is cooked on a stove or an open fire and the approach used if any to channel the smoke, i.e. a chimney or a hood or both. You may need to probe for an exact description of the type of stove. |
| 77 | Is cooking done inside the house, outside the house, or both? | The purpose of this question is to collect information on the location where food is prepared in the household: in the household, outside the household, or both. This information is important in providing an indicator of the air quality inside and around the dwelling. |
| 78 | *Main material of the floor (observation)* | This is an observation not a question since you will usually be able to see for yourself what kind of floor the house has. However, ask if you are not sure. If there is more than one kind of flooring material, record the main type of material (the material that covers the largest amount of floor space). |
| 79 | *Main material of the roof (observation)* | As with the floor material, you will usually be able to see for yourself what kind of roof material the house has. However, observing the roof material may not always be easy or you may be able to observe part but not the whole roof. Ask the respondent if you are not sure or if you cannot observe the roof properly. If the household lives in an apartment building, look at the roof from a reasonable distance and ask the respondent if necessary. If there is more than one kind of roofing material, record the main type of material (the material that covers the largest amount of roof space). |
| 80 | *Main material of the exterior walls (observation)* | This is not a question but an observation. You will usually be able to see for yourself what kind of material the exterior walls are made of. However, ask the respondent if you are not sure. Again, if there is more than one kind of wall material, record the main type of material (the material that covers the largest amount of wall space). |
| 81 | Do you have a separate room which is used as a kitchen? | This question seeks information on whether the household has a separate room used as a kitchen in order to provide additional information on the hygiene status of the household. |
| 82 | Which of the following is the primary way you distinguish members of your population? | This question asks about the *primary* method for distinguishing between members of the population. While more than one of these categories might apply, emphasize that you would like to know which of the categories is used most often. For questions 83-87, only ask the question that corresponds to the response on question 82. For example, if the respondent states that *language spoken (04)* is the primary way of distinguishing members of her/his population, then only ask question 86. Do not ask questions 83, 84, 85, or 87. |
| 83 | *If caste (01) is the response for question 82:* What caste are you a member of? | Only ask this question if the response to question 82 was *caste (01).* |
| 84 | *If race (02) is the response for question 82:* Which racial group are you a member of? | Only ask this question if the response to question 82 was *race (02).* |
| 85 | *If tribe (03) is the response for question 82:* Which tribal group are you a member of? | Only ask this question if the response to question 82 was *tribe (03).* |
| 86 | *If language spoken (04) is the response for question 82:* What language do you speak in your household? | Only ask this question if the response to question 82 was *language spoken (04).* |
| 87 | *If religion (05) is the response for question 82:* What religion do you practice in your household? | Only ask this question if the response to question 82 was *religion (05).* |
| 88 | What is the average monthly income for the entire household? | This question asks about the average monthly income in the household. In most cases this amount will be an estimate as most households may not receive a regular income. Encourage the respondent to include monetary income from all household members. Enter the amount in the top row given for responses, and the letter associated with the local currency in the box next to *(currency)* on the bottom row. |

**FSQ—Food Security Questionnaire**

**I. Purpose**

The Food Security Questionnaire (FSQ) will be implemented at each household at the 60 month study visits. This is an optional collection, but highly recommended for sites to use. The FSQ should be administered at the same time as the SES Assessment Form (FSE). These questions can be answered by the mother of the child, the child’s primary caregiver, or the head of household. However, it is important that the same person respond each time this questionnaire is administered.

**II. Material**

Food Security Questionnaire Form (FSQ), pen and clipboard.

**III. Methods**

1. Each of the questions in the Food Security section is asked with a recall period of four weeks (30 days). The respondent is first asked a frequency-of-occurrence to determine whether the condition happened rarely (once or twice), sometimes (three to ten times) or often (more than ten times) in the past four weeks.
2. Some of the nine questions inquire about the respondents’ perceptions of food vulnerability or stress (e.g., did you worry that your household would not have enough food?) and others ask about the respondents’ behavioral responses to insecurity (e.g., did you or any household member have to eat fewer meals in a day because there was not enough food?). The questions address the situation of all household members and do not distinguish adults from children or adolescents. All of the questions ask whether the respondent or other household members either felt a certain way or performed a particular behavior over the previous four weeks.
3. The questionnaire should be asked in its entirety. Project staff should avoid picking and choosing only certain questions. Research has shown that the complete set of questions does a better job of distinguishing the household food insecurity (access) level than any question on its own.
4. Question Guidance

|  |  |  |
| --- | --- | --- |
| # | Question | Response |
|  | Participant ID | Write the child’s participant ID in the space provided at the upper left corner. |
| 1 | Study Researcher / Nurse / Fieldworker ID | Enter the Study Researcher / Nurse / Fieldworker’s unique ID number here. |
| 2 | Today’s date | Format DD/MMM/YY |
| 3 | In the past four weeks, did you worry that your household would not have enough food? | This question asks the respondent to report their personal experience with uncertainty and anxiety about acquiring food during the previous month. The interviewer should explain that household is defined as the people who usually sleep in the household. Mention that this definition of household applies to all the questions with that term. |
| 4 | In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources? | One domain of food insecurity (access) is having limited choices in the type of food that a household eats. This question asks whether any household member was not able to eat according to their preference due to a lack of resources. Preference can refer to the form of a particular food (i.e., whole rice vs. broken rice), type of staple (i.e., millet vs. corn) or a high quality food (i.e., a piece of meat or fish). Preferred foods may or may not be nutritionally high quality. The interviewer should also read the definition of a “lack of resources.” Mention that this definition of household applies to all the questions with that term. The respondent needs to answer on behalf of all household members |
| 5 | In the past four weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources? | This question asks about dietary choices related to variety – i.e., whether the household had to eat an undesired monotonous diet (little diversity in the different types of foods consumed). The respondent needs to answer on behalf of all household members. |
| 6 | In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food? | This question, which also captures the dimension of limited choices, asks whether any household member had to eat food that they found socially or personally undesirable due to a lack of resources. Often these are foods or food preparations that are consumed only under hardship. Different people may consider different foods to be undesirable, so it is best not to provide examples here at first. The respondent needs to answer on behalf of all household members, according to his or her own perception of the types of food household members ate during the previous four weeks. For all questions, it is important to remind respondents that the examples are not an exhaustive list. |
| 7 | In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food? | This question asks whether the respondent felt that the amount of food (any kind of food, not just the staple food) that any household member ate in any meal during the past four weeks was smaller than they felt they needed due to a lack of resources. The respondent should answer according to his or her perception of what constitutes enough food for the needs of the household members. The respondent needs to answer on behalf of all household members. |
| 8 | In the past four weeks, did you or any other household member have to eat fewer meals in a day because there was not enough food? | This question asks whether any household member, due to lack of food, had to eat fewer meals than the number typically eaten in the food secure households in their area. The respondent needs to answer on behalf of all household members. |
| 9 | In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food? | This question asks about a situation in which the household has no food to eat of any kind in the home. This describes a situation where food was not available to household members through the households’ usual means (e.g., through purchase, from the garden or field, from storage, etc.). |
| 10 | In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food? | This question asks whether the respondent felt hungry at bedtime because of lack of food or whether the respondent was aware of other household members who were hungry at bedtime because of lack of food. The respondent needs to answer on behalf of all household members. |
| 11 | In the past four weeks, did you or any household member go a whole day and night without eating anything because there was not enough food? | This question asks whether any household member did not eat from the time they awoke in the morning to the time they awoke the next morning due to lack of food. The respondent needs to answer on behalf of all household members. |