**Protocol for Specimen Collection, Field Testing, Specimen Processing, Storage, and Shipment**

National Micronutrient Status Survey

Nepal 2016

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# Overall Universal Precautions and Considerations

* Collection, testing, and processing of biological specimens are critical parts of the Nepal National Micronutrient Status Survey (NMSS).
* Specimen collection, processing, transport, and storage must be done with great care so that the laboratory results are valid and accurately reflect the micronutrient status of the survey subjects.
* **Universal precautions** are procedures that must be followed by all team members to prevent exposure to HIV, hepatitis, and other infectious agents that are encountered during all collection, processing and handling of biological specimens.
* ALL specimens should be considered POTENTIALLY INFECTIOUS. Practice of universal precautions (**Annex 1**) throughout specimen collection, transport, processing, storage, and shipment.

# Overview: Cold Chain Logistics

It is essential that proper cold chain logistics (**Annex 2**) are followed throughout the survey. Cold chain follows biological specimens from initial collection until the specimen is analyzed. All team members should be aware of cold chain logistics.

*Portable Freezers and Cold Boxes*

* Portable freezers and cold boxes (**Figure 1**) will be used in the field to ensure the proper cold chain of the specimens.

**Figure 1. Portable Freezer and Cold Box**



* A cold box with a handle will be provided to each Laboratory Technician, Phlebotomist, and Pathologist for ease in transport of specimens in the field. Additional Styrofoam cold boxes will be made available to the Laboratory Technicians and Pathologists if extra cold boxes are needed.
* Frozen gel packs will be used in the field to keep specimens cool after specimen collection in the household and frozen after processing in the field.
  + It can take up to 48 hours to initially freeze gel packs until hard. Be sure to place enough gel packs into a ≥-20°C freezer prior to the start of the survey.
  + Extra frozen gel packs will be available in a -20°C located in the portable freezers (and district Public Health Centers) so that enough frozen gel packs are available each day.
    - Be sure to refreeze gel packs which are used during the day so that there will be a constant supply of frozen gel packs available each day.
* Cold boxes are used in the field to store specimens after specimen collection in the household and prior to specimen processing.
* Each cold box will contain (5-6) frozen gel packs, (1) digital thermometer, (1) vacutainer rack or cryovial box for specimens, and a small strip of bubble wrap which should be placed between the frozen gel packs and the vacutainer rack/cryovial box to prevent freezing of the specimens.

* Portable freezers are used in the field to keep gel packs frozen and to store specimens after they have been processed.
  + Each portable freezer will contain frozen gel packs and processed specimens. Each Laboratory Technician will have a portable freezer.

***Essentials of Cold Chain Logistics***

* **BLOOD, URINE, and STOOL SPECIMENS SHOULD NEVER BE FROZEN OR PLACED INTO THE PORTABLE FREEZERS UNTIL THEY ARE PROCESSED**.
  + Blood, urine, and stool specimens should **ONLY** be stored in a cold box containing a few frozen gel packs until they have been centrifuged and transferred to cryovials. Do not allow specimens to touch frozen gel packs directly because they might freeze.
* Only open lid to cold box when placing new specimens into the cold box or when replacing thawing gel packs.
  + When you open the cold box, record the temperature of the inside of the cold box on the “**Specimen Control Forms A, B, & C**” (**Annexes 3a, 3b and 3c**).
  + The temperature inside the cold box should always remain <8°C.
  + The Phlebotomist, Laboratory Technician, and Pathologist should replace thawing gel packs with frozen gel packs when temperature is ~6°C.
    - The Laboratory Technician will have the frozen gel packs with them in the portable freezer. The Phlebotomists can exchange thawing gel packs with frozen gel packs with the deliver specimens to the Laboratory Technician for processing. The Pathologist will be working in the same area as the Laboratory Technician, so they can replace thawing gel packs when needed.
* At the end of each day, the Laboratory Technician ensures that all gel packs are placed into the -20ºC freezer in the evening so that they are frozen until hard and available to use in the cold boxes during the next day’s field use.
  + Additional frozen gel packs will be available at the district Public Health Center.
  + Processed specimens should be placed into a -20°C freezer for storage until shipped/transported to Kathmandu.
* Liquid controls for the HemoCue 301 analyzers and the vitamin A2 stock for MRDR dosing should be stored in a 1°C - 6°C refrigerator or cold box with frozen gel packs during the survey.
* To ensure the proper transport of biological specimens, vitamin A2 stock, and liquid controls, procedures for cold chain logistics need to be maintained to avoid adverse effects on specimen results (see **Table 1** below).

**Table 1: Cold Chain Logistics**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Household** | **Field (Processing)** | **Vehicle** | **Laboratory (Storage)** | **DHL/World**  **Courier** | **Laboratory (Analysis)** |
| **Procedure** | Blood collection & Vitamin A2 doses  Field testing  Urine collection and processing (can be done in field)  Stool collection | Centrifuge Blue Top, Red Top, and Purple Top Vac  Process stool specimens using Kato Katz and *H. pylori* | Transport to Laboratory (processed specimens)  Transport BD Purple Tops (for blood disorders) weekly to the Laboratories in Kathmandu | Storage of specimens (freezer), vitamin A2 stock, and liquid controls (refrigerator or cold box with frozen gel packs) | Transport to Germany, China, and Guatemala | Germany: ELISA  Peking University:  RBC Folate, UI  Guatemala (INCAP):  MRDR/Retinol, UI, B12, Zinc  NPHL/Samyak Lab:  CBC and Blood Disorders  AFRIMS:  Stool for *H. pylori* |
| **Packaging** | Cold Box | Portable Freezer | Cold Box and Portable Freezer | Refrigerator/  Freezer | Packed on dry ice for shipment via DHL or World Courier | Freezers |
| **Temperature** | < 8°C (Replace thawing gel packs at ~6°C) | -10 🡪 -20ºC | < 8°C (Replace thawing gel packs at ~6°C) for cold box and -10 🡪 -20ºC for Portable Freezer | 1-6°C (refrigerator)  -10 🡪 -20ºC /-70ºC  (freezer) | <-10°C | ≥-70°C |
| **Cold Chain** | Store blood specimens and vitamin A2 in cold boxes that are maintained with frozen gel packs (replace thawing gel packs with frozen gel packs as needed)  Transfer urine into labelled cryovials and place into cold box | Transfer serum and/or plasma into labelled cryovials and place in cyrovial boxes inside the Portable Freezer  Transfer urine specimens into Portable Freezer | Add new frozen gel packs before transport | 1-6°C (refrigerator)  -10 🡪 -20ºC /-70ºC  (freezer) | Cold Box | ≥-70ºC freezer |
| **Verification method** | Digital Thermometer | Digital Thermometer | Digital Thermometer | Thermometer | Thermometer | Thermometer |

# Overview: Responsibilities of Laboratory Personnel

* There are several laboratory personnel involved in the survey including, Phlebotomists, Laboratory Technicians, Pathologists, Laboratory Coordinators, National Level Ministry of Health Coordinator, and the CDC Laboratory Representative.
* The Phlebotomist is responsible for:
  + Specimen collection and testing in the household (**Annex 5: Flow Chart for Specimen Field Testing and Field Processing**)
  + Maintenance of the cold chain in the field (**Annex 2**)
  + Organization of supplies needed for specimen collection in the field every day (**Annex 4a**).
    - Each day enough supplies need to be available for a total of 4 households per day (plus 10% extra supplies).
* Recording participant label and specimen information on the “**Specimen Control Form A**” (**Annex 3a**).
* The Laboratory Technician is responsible for:
  + Processing all the blood and urine specimens (in the field and laboratory) (**Annex 5: Flow Chart for Specimen Field Testing and Field Processing**)
  + Organization of supplies needed for specimen collection in the field every day (**Annex 4b**).
    - Each day enough supplies need to be available for a total of 12 households per day (plus 10% extra supplies).
  + Set a clean working area to process specimens.
  + Recording participant label and specimen information on the “**Specimen Control Form B**” (**Annex 3b**).
  + Maintenance of the cold chain in the field (**Annex 2**).
  + Responsible for storage of specimens while in the field and assisting the Laboratory Coordinator in transporting specimens to the laboratory.
  + Assist the Laboratory Coordinator to transfer of BD vacutainers (Blood Disorders) to Kathmandu for analysis.
* The Pathologist is responsible for:
  + Processing all the stool specimens (in the field- **Annex 5: Flow Chart for Specimen Field Testing and Field Processing**) using the Kato Katz Procedure (**Annex 22**)
  + Organization of supplies needed for specimen collection in the field every day (**Annex 4c**).
    - Each day enough supplies need to be available for a total of 12 households per day (plus 10% extra supplies).
  + Recording participant label and specimen information on the “**Specimen Control Form C**” (**Annex 3c**).
  + Maintenance of the cold chain in the field (**Annex 2**).
  + Responsible for stool specimens until they are transferred to Kathmandu for analysis.
  + Assist the Laboratory Coordinator in transporting stool specimens to Kathmandu.
* The National Level Ministry of Health Coordinator is responsible for:
  + Storing specimens in the National Public Health Laboratory at -86°C.
  + Assisting the Laboratory Coordinators with shipping the specimens to laboratories in other countries.
* The Laboratory Coordinator and CDC Laboratory Representative are responsible for:
  + Laboratory and field work planning.
  + Training.
  + Quality control oversight.
  + Ensure cryovial boxes are properly labelled by Laboratory Technicians and sorted according to specimen type and age group
  + Daily transfer of stool and BD (Blood Disorders) Purple Top specimens to Kathmandu.
  + Transport of specimens in the field to the central public health laboratories in each district.
  + Transport of specimens and maintenance of cold chain between the labs in the districts and the National Public Health Laboratory in Kathmandu.
  + Shipping the specimens to labs outside the country for analysis.
  + Overall support and supervision of all laboratory procedures.

# Overview: Labelling Procedures

**Always read labels carefully before affixing them to anything. Match the survey participant to the label and to the correct specimen or box to be labeled.**

***Labelling for Household Questionnaires and Food Specimens***

Enumerators will be responsible for labeling the questionnaires and food specimens. However, Phlebotomists may be asked to assist with the collection and storage of food specimens, so it is important to know the different labels for this purpose.

* **HH Questionnaire** (1)- 1 for HH questionnaire (labels for other questionnaires will come with their respective age groups)
* **Salt Sample** (2)- one for Ziploc bag and one for paper bag
* **Wheat Flour Sample** (2)- one for Ziploc bag and one for paper bag
* **Extra Labels** (1)

***Labelling of Biological Specimens***

Each age group will be assigned their own set of labels because different specimens will collected/processed for each. The different age groups include:

* Children 6-59 months
* Children 6-9 years
* Adolescent girls 10-19 years
* Adolescent boys 10-19 years
* Non-pregnant Women 15-49 years
* Pregnant Women 15-49 years

Each **Child 6-59 months** will have a set of 27 large labels (Figure 2) and 1 small label (for 0.2mL PCR tube) (Figure 3). Not all labels will be used for all children.

* **Questionnaire** (1)
* **Specimen Control** (3)- 1 for Phlebotomist, 1 for Lab Technician & 1 for Pathologist
* **Blue Top Vac** (1)
* **Purple Top Vac** (1)
* **BD Purple Top** (1)
* **Malaria Ref Log** (1)
* **V Leish Ref Log** (1)
* **Hgb Ref Log** (1)
* **Stool Cup 1** (1)- for Kato Katz and *H. pylori*
* **MRDR Purple Top** (1)
* **Serum Vial** (2) -1 for analysis and 1 for backup
* **RBC Folate** (2) - 1 for analysis and 1 for backup
* **Plasma Vial** (1) - 1 for backup
* **MRDR Vial** (2) - 1 for analysis and 1 for backup
* **Kato Katz Slide** (2)- for duplicate readings
* **Stool Vial** (1)- 1 for analysis of *H. pylori*
* **Extra Labels** (5)

Each **Child 6-9 years** will have a set of 9 large labels (Figure 2).

* **Questionnaire** (1)
* **Specimen Control** (2)- 1 for Phlebotomist and 1 for Lab Technician
* **Urine Cup** (1)
* **Urine Vial** (2) -1 for analysis and 1 for backup
* **Extra Labels** (3)

Each **Adolescent Girl 10-19 years** will have a set of 15 large labels (Figure 2) and 1 small label (for 0.2mL PCR tube) (Figure 3).

* **Questionnaire** (1)
* **Specimen Control** (2)- 1 for Phlebotomist and 1 for Lab Technician
* **Red Top Vac** (1)
* **Purple Top Vac** (1)
* **Malaria Ref Log** (1)
* **Hgb Ref Log** (1)
* **H. pylori Ref Log** (1)
* **Serum Vial** (1)- 1 for backup
* **RBC Folate** (2) - 1 for analysis and 1 for backup
* **Plasma Vial** (1) - 1 for backup
* **Extra Labels** (2)

Each **Adolescent Boy 10-19 years** will have a set of 15 large labels (Figure 2) and 1 small label (for 0.2mL PCR tube) (Figure 3).

* **Questionnaire** (1)
* **Specimen Control** (2)- 1 for Phlebotomist and 1 for Lab Technician
* **Red Top Vac** (1)
* **Purple Top Vac** (1)
* **Malaria Ref Log** (1)
* **Hgb Ref Log** (1)
* **H. pylori Ref Log** (1)
* **Serum Vial** (1)- 1 for backup
* **Plasma Vial** (1) - 1 for backup
* **Extra Labels** (4)

Each **Non-Pregnant Woman 15-49 years** will have a set of 30 large labels (Figure 2) and 1 small label (for 0.2mL PCR tube) (Figure 3). Not all labels will be used for all women.

* **Questionnaire** (1)
* **Specimen Control** (3)- 1 for Phlebotomist, 1 for Lab Technician & 1 for Pathologist
* **Blue Top Vac** (1)
* **Purple Top Vac** (1)
* **BD Purple Top** (1)
* **Urine Cup** (1)
* **Malaria Ref Log** (1)
* **V Leish Ref Log** (1)
* **Hgb Ref Log** (1)
* **Stool Cup** (1)- 1 for Kato Katz and *H. pylori*
* **MRDR Purple Top** (1)
* **Serum Vial** (2) -1 for analysis and 1 for backup
* **RBC Folate** (2) - 1 for analysis and 1 for backup
* **Plasma Vial** (1) - 1 for backup
* **Urine Vial** (2) – 1 for analysis and 1 for backup
* **MRDR Vial** (2) - 1 for analysis and 1 for backup
* **Kato Katz Slide** (2)
* **Stool Vial** (1)- 1 for analysis of *H. pylori*
* **Extra Labels** (5)

Each **Pregnant Woman 14-59 years** will have a set of 15 large labels (Figure 2) and 1 small label (for 0.2mL PCR tube) (Figure 3). Not all labels will be used for all women.

* **Questionnaire** (1)
* **Specimen Control** (2)- 1 for Phlebotomist and 1 for Lab Technician
* **Red Top Vac** (1)
* **Purple Top Vac** (1)
* **Urine Cup** (1)
* **Malaria Ref Log** (1)
* **Hgb Ref Log** (1)
* **Serum Vial** (1) - 1 for backup
* **Plasma Vial** (1) - 1 for backup
* **Urine Vial** (2) – 1 for analysis and 1 for backup
* **Extra Labels** (3)
* The Enumerators are responsible for:
  + Putting the “**Questionnaire**” labels on the questionnaires at the start of the interview.
* The Phlebotomist is responsible for:
  + Placing the “**Blue Top Vac**”, “**Purple Top Vac**”, “**BD Purple Top**”, “**Red Top Vac**”, “**Urine Cup**”, “**Stool Cup**”, and “**MRDR Purple Top**” labels on the corresponding vacutainers, cups, and vials.
  + Placing the “**Specimen Control”, “Malaria Ref Log”**, “**V Leish Ref Log**”, “**Hgb Ref Log**” and “**H. pylori Ref Log**” labels onto the corresponding forms.
* The Laboratory Technician is responsible for:
  + Placing the “**Serum Vial**”, “**Plasma Vial**”, “**RBC Folate**”, “**Urine Vial**”, and “**MRDR Vial**” labels on the cryovials and processing.
  + Placing the “**Specimen Control”** label on the corresponding form.
  + Placing the small label on the 0.2mL PCR tube (**Figure 3**) after processing.
* The Pathologist is responsible for:
  + Placing the “**Kato Katz Slide**” labels on the corresponding microscope slides.
  + Placing the “**Stool Vial 1**” label on the corresponding cryovial.
  + Placing the “**Specimen Control”** label onto the corresponding form.

**Figure 2**: **Example of labels used in the field and laboratory for each age group and household**

**Household Labels:**



Extra Labels

Enumerator Labels

**Children 6-59 Months:**



Extra Labels

Pathologist Labels

Lab Technician Labels

Phlebotomist Labels

Enumerator Label

**Children 6-9 Years:**



Extra Labels

Lab Technician Labels

Phlebotomist Labels

Enumerator Label

**Adolescent Boys 10-19 Years:**



Extra Labels

Lab Technician Labels

Phlebotomist Labels

Enumerator Label

**Adolescent Girls 10-19 Years:**



Extra Labels

Lab Technician Labels

Phlebotomist Labels

Enumerator Label

**Non-Pregnant Women 15-49 Years:**



Extra Labels

Pathologist Labels

Lab Technician Labels

Phlebotomist Labels

Enumerator Label

**Pregnant Women 15-49 Years:**



Lab Technician Labels

Extra Labels

Phlebotomist Labels

Enumerator Label

Laboratory Technician Labels

Extra Labels- Can be used by Enumerator, Phlebotomist, Laboratory Technician, or Pathologist

***Extra Labels***

* Each set of labels will have some addition “Extra” labels. These can be used in cases where labels may have been torn or damaged.
* Write the sample description on the label using a marker pen.

***PCR Tube Labels***

* In addition to these large labels, there is also a small white label for the 0.2mL PCR tube.
* The small white PCR labels come on a large sheet of many labels (**Figure 3**).
* The correct corresponding label should be used to label the PCR tube so that the number on the large labels matches the number on the small label for each child.

**Figure 3**: **Small white label for 0.2mL PCR tubes**



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***Labelling of Cryovial Boxes***

* While in the field, Laboratory Technicians sort labelled cryovials into individual boxes and label the specimen type and age group (e.g., “Serum Vial 1- Child 6-59 mo”) on the outside of the box.
* Cryovial boxes will have their own sets of labels (**Figure 4**).

**Figure 4**: **Labelling of Cryovial Boxes**











* Each box will be labelled with 3 box labels:
  + One label goes on the top of the box lid, another goes on the side of the box lid, and the last goes on the side of the bottom of the box (**Figure 4**).
  + The Laboratory Coordinator is responsible for ensuring cryovial boxes are properly labelled and specimens properly sorted into cryovial boxes by the Laboratory Technicians.
* Cryovial boxes will then be numbered numerically so that the specimen inside the box can be assigned to a specific cryovial box for the specimen inventory.
  + Box numbers however will not be assigned until all specimens are transferred to the NPHL.
* At the NPHL, the Laboratory Coordinators will work together to assign box numbers. The box numbers should not be duplicated (e.g., one box will receive Box #1, the second box will receive Box #2, etc.). The box number must then be recorded on the “**Specimen Control Form B**” (**Annex 3b**).
  + A specimen inventory will also need to be generated by the Laboratory Coordinators. A specimen inventory will include each specimen ID within a specific box.
    - The specimen inventory will be sent along with the specimens to each laboratory.
* Labelled 0.2mL PCR tubes will be stored in a PCR tube cryovial box for easy storage.
  + The 0.2mL PCR tubes can be consolidated from the teams at the end of each day into one PCR tube cryovial box.
  + The cryovial boxes containing the labelled 0.2mL PCR tubes will then be shipped to Germany for analysis.
* All specimens will be in a -86°C freezer at the National Public Health Laboratory in Kathmandu until shipped for analysis or stored long-term as backup specimens.

**REMEMBER: Read the labels carefully before affixing them to anything.**

**Match the survey participant to the label and to the correct specimen to be labeled.**

# Procedures for Specimen Collection in the Household

***Duties Prior to Specimen Collection:***

* The Phlebotomist should pack the backpack with all the supplies needed for daily field use (**Annex 4a**).
* Prepare 7 MRDR doses (4 for children 6-59 months and 3 for non-pregnant women 15-49 years) for the day as instructed by the Laboratory Coordinator (or Enumerator/Supervisor).
* Check the quality control of the HemoCue 301 using the liquid controls (**Annex 16**).

***Specimen Types***:

* The Phlebotomist will be responsible for collecting several types of specimens, including blood, urine, and stool.
* The type of specimens collected will depend on the age group.
  + Children 6-59 months🡪 Blood and Stool
  + Children 6-9 years🡪 Urine
  + Adolescent girls 10-19 years🡪 Blood
  + Adolescent boys 10-19 years🡪 Blood
  + Non-pregnant Women 15-49 years🡪 Blood, Urine, and Stool
  + Pregnant Women 15-49 years🡪 Blood and Urine

***Venous Blood Collection:***

* Obtain informed consent for blood collection
* Set up all the supplies needed for the blood collection. This requires a comfortable location with a flat surface sufficiently large to lay out the absorbent pad and all equipment and supplies prior to collecting the venous sample from the participant.
* For small children, have the mother or caretaker hold the child.
* Collect the venous blood specimen (**Annex 6: Procedure for Venous Blood Collection**). Vacutainers are used for venous blood collection (**Figure 5**):

**Figure 5: Types of Vacutainers**

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* The amount and number of vacutainers of blood collected will depend on the age group.
* Children 6-59 months🡪 1 Blue Top Vacutainer (zinc, B12, and ELISA) and 2 Purple Top Vacutainers (1 for Hb, malaria, and visceral leishmaniasis and 1 for blood disorders)
* Adolescent girls 10-19 years🡪 1 Purple Top Vacutainer (Hb, malaria, and H. pylori) and 1 Red Top Vacutainer (ELISA)
* Adolescent boys 10-19 years🡪 1 Purple Top Vacutainer (Hb, malaria, and H. pylori) and 1 Red Top Vacutainer (ELISA)
* Non-pregnant Women 15-49 years🡪 1 Blue Top Vacutainer (zinc, B12, and ELISA) and 2 Purple Top Vacutainers (1 for Hb, malaria, and visceral leishmaniasis and 1 for blood disorders)
* Pregnant Women 15-49 years🡪 1 Purple Top Vacutainer (Hb and malaria) and 1 Red Top Vacutainer (ELISA)
  + Four randomly selected children 6-59 months and three non-pregnant women 15-49 years from each cluster will be selected to provide an additional purple top vacutainer for MRDR. Collection will occur the first day in each cluster.
  + For children 6-59 months and non-pregnant women 15-49 years, the Phlebotomist will need to collect 3mL blood into each **Purple Top Vacutainer** and 5mL blood in each **Blue Top Vacutainer**, and mix the specimens gently.
  + For pregnant women 15-49 years, adolescent girls and adolescent boys, the Phlebotomist will collect 3mL blood into each **Purple Top Vacutainer** and 2mL blood in each **Red Top Vacutainer**.
* The Phlebotomists will have only two opportunities to collect blood from each participant.
* The vacutainers should then be labelled with the correct corresponding participant’s label. The Phlebotomist should also place the participant’s label on the “**Specimen Control Form A**” for Phlebotomists (**Annex 3a**).
* All waste obtained in the household should be appropriately disposed of using sharps containers (i.e., needles) and autoclave bags (i.e., gauze, alcohol swabs, kimwipes, gloves, etc.) (**Annex 1: Universal Precautions**).
* All vacutainers should be stored in a vacutainer rack or cryovial box inside the cold box. Be sure that the vacutainers are separated from the frozen gel packs using bubble wrap.
* Do not let the vacutainers come in direct contact with the frozen gel packs because they might freeze and will be ruined!
* Record the temperature of the cold box on the “**Specimen Control Form A**” when placing specimens inside the cold box (**Annex 3a**).
* For the subsample of children 6-59 months and non-pregnant women 15-49 years selected for MRDR, the Phlebotomist will return to the participant’s home 4 hours after dosing with vitamin A2 to collect a third blood sample (3mL blood in a third **Purple Top Vacutainer**), and mix the specimen gently.
  + Label the vacutainer with the “**MRDR Purple Top**” label.
  + Find the appropriate participant’s number on the “**Specimen Control Form A**” (**Annex 3a**) and mark collected for MRDR specimens.
  + Procedures for MRDR dosing and specimen collection are listed below in “Specimen Field Testing.”

***Urine Collection:***

* The Phlebotomist will collect urine from the following age groups in each household:
* Children 6-9 years
* Non-pregnant Women 15-49 years
* Pregnant Women 15-49 years
* The Phlebotomist should provide each participant with a labelled urine cup and provide specific instructions (**Annex 20: Procedure for Urine Collection in the Field**) regarding the urine collection process.
* Each participant should provide a minimum of 6mL of urine.

* If a participant is unable to provide a urine sample before the team leaves the household, then the Phlebotomist should recommend that the participant drink some water so that the participant might be able to provide a sample.
* All urine cups should be stored inside the cold box. Be sure that the urine cups are separated from the frozen gel packs using bubble wrap.
  + Record the temperature of the cold box on the “**Specimen Control Form A**” when placing specimens inside the cold box (**Annex 3a**).

***Stool Collection:***

* The Phlebotomist will collect stool from the following age groups in each household:
* Children 6-59 months
* Non-pregnant Women 15-49 years
* The Phlebotomist should provide each participant with a stool collection kit containing a stool collection container, a disposable wooden spatula or spoon, and one pre-labelled stool cup.
* The Phlebotomist should instruct each participant on the collection and transfer of stool to the cup as well as proper storage to the stool specimens until they are picked up by the teams (**Annex 21: Procedure for Stool Specimen Collection**).
* Each participant should provide a minimum of 3 g of stool.
* The Phlebotomist (or another team member) will pick up the stool specimens from each household the following day.
  + Find the appropriate participant’s number on the “**Specimen Control Form A**” (**Annex 3a**) and mark collected for stool (Kato Katz and *H. pylori*).
  + Place the specimen inside a Ziploc bag and store them inside a cold box containing frozen gel packs until they are transferred to the Pathologist for processing.

***Transfer of Specimens to the Laboratory Technician and Pathologist:***

* Transfer all vacutainers and urine and stool specimens to the Laboratory Technician after each household.
* Be sure to transfer the remaining labels to the Laboratory Technician (Figure 2) for each participant along with the specimens.
  + The Laboratory Technician will be sure the Pathologist receives stool specimens and the necessary labels for the Kato Katz microscope slides and H. pylori vials.
* The Phlebotomist should complete the “**Specimen Transfer Form**” (**Annex 3d**) to include the sample IDs and time of blood collection, urine collection and stool collection (urine and stools may be collected later, so the time of pickup will be sufficient). This form will be given to the Laboratory Technician when specimens are transferred.

# Specimen Field Testing

The Phlebotomists are responsible for malaria and Visceral Leishmaniasis testing (only children 6-59 mos and non-pregnant women 15-49 yrs), hemoglobin measurement, H. pylori testing (only adolescent girls and boys), and MRDR dosing and specimen collection in the field.

The **Purple Top Vacutainer** labelled **“Purple Top Vac”** will be used for malaria testing, Visceral Leishmaniasis testing (only children 6-59 mos and non-pregnant women 15-49 yrs), hemoglobin measurement, and H. pylori testing (only for adolescent girls and boys). The second **Purple Top Vacutainer** labelled **“BD Purple Top”** (only for children 6-59 months and non-pregnant women 15-49 years) should not be used because this vacutainer is for analysis of blood disorders.

***Malaria Testing:***

* Malaria should be checked using a rapid malaria antigen (HRP2/pLDH) test kit for *Plasmodium falciparum* and *P. vivax* (**Annex 7; Procedure for Checking Malaria Using the Rapid Malaria Antigen (HRP2/pLDH) Combo Test Kit**).
  + Results take 20 minutes, so it is important to start the malaria test prior to measuring the hemoglobin.
  + Use a timer so that the 20 minutes can be monitored and the results are taken in the correct amount of time.
* Once the test is complete, record the result on the questionnaire and inform the participant of the result.
* For participants who test positive for either *P. falciparum* or *P. vivax*, the Phlebotomist should complete a “**Malaria Status and Referral Slip**” (**Annex 8**) and refer him/her to the local health center.
  + Place the participant’s label on the “**Referral Log for Participants Who Test Positive for Malaria**” (**Annex 9**).
  + Be sure to designate which form(s) of malaria the participant tested positive.
  + Complete these forms daily to ensure all participants are identified as having malaria.
  + Give these logs to the Laboratory Coordinator at the end of each day.

***Visceral Leishmaniasis Testing (Children 6-59 mo and Non-Pregnant Women 15-49 yrs ONLY):***

* Visceral Leishmaniasis should be checked using a rapid diagnostic test using the rK39 antigen (**Annex 10: Procedure for Checking Visceral Leishmaniasis Using the IT LEISH Rapid Test Kit**).
  + Results take 20 minutes, so it is important to start the Visceral Leishmaniasis test at the same time as the malaria test and prior to measuring the hemoglobin.
  + Use a timer so that the 20 minutes can be monitored and the results are taken in the correct amount of time.
* Once the test is complete, record the result on the questionnaire and inform the participant of the result.
* For participants who test positive, the Phlebotomist should complete a “**Visceral Leishmaniasis Status and Referral Slip**” (**Annex 11**) and refer him/her to the local health center.
  + Place the participant’s label on the “**Referral Log for Participants Who Test Positive for Visceral Leishmaniasis**” (**Annex 12**).
  + Complete these forms daily to ensure all participants are identified as having malaria.
  + Give these logs to the Laboratory Coordinator at the end of each day.

***Hemoglobin Measurement:***

* While waiting on the results from the malaria and Visceral Leishmaniasis tests, the Phlebotomist should proceed to measuring the hemoglobin using the HemoCue® Hb-301photometer (**Annex 13**).

* Quality control of the HemoCue 301 operation is performed at the beginning of each day using three levels of liquid controls (i.e., Levels 1, 2, and 3).
* Results are recorded for each liquid controls on the “H**emoCue® Hemoglobin Quality Control Form**” (**Annex 16**). Be sure to store liquid controls in a refrigerator (1-6°C) or in a cold box with frozen gel packs when not in use so the controls stay cool.
* Once hemoglobin has been measured, the Phlebotomist should record the result on the questionnaire and inform the participant of the result.
* The hemoglobin level will vary depending on age. Below are the hemoglobin cut-offs:
  + Children 6-59 months🡪 <11.0 g/dL
  + Children 5-11 years🡪 <11.5 g/dL
  + Children 12-14 years🡪 <12.0 g/dL
  + Non-pregnant Women 15-49 years🡪 <12.0 g/dL
  + Pregnant Women 15-49 years🡪 <11.0 g/dL
  + Men ≥ 15 years🡪 <13.0 g/dL
* If the participant has a hemoglobin level indicating they have severe anemia, then the participant should be provided with a “**Hemoglobin Status and Referral Slip**” (**Annex 14**) to refer the participant to the local health center.
* Place the child’s label on the “**Referral Log for Participants Who Are Anemic**” (**Annex 15**).
* Complete these forms daily to ensure all participants with anemia are identified as having anemia.
* Give these logs to the Laboratory Coordinator at the end of each day.

***H. pylori Testing (Adolescent Girls and Boys ONLY):***

* H. pylori should be checked using the QuickVue rapid test kit (**Annex 17; Procedure for Checking H. pylori using the QuickVue Rapid Test Kit**).
  + Results take about 5 minutes, so it is important to start the H. pylori test after the malaria test, but prior to measuring the hemoglobin.
  + Use a timer so that the 5 minutes can be monitored and the results are taken in the correct amount of time.
  + Once the test is complete, record the result on the questionnaire.
* For participants who test positive for either *H. pylori*, the Phlebotomist should complete a “**H. pylori Status and Referral Slip**” (**Annex 18**) and refer him/her to the local health center.
  + Place the participant’s label on the “**Referral Log for Participants Who Test Positive for H. pylori**” (**Annex 19**).
  + Be sure to designate which form(s) of malaria the participant tested positive.
  + Complete these forms daily to ensure all participants are identified as having malaria.
  + Give these logs to the Laboratory Coordinator at the end of each day.

***MRDR Dosing and Specimen Collection:***

* The following age groups in each household will participate in MRDR dosing and specimen collection:
* Children 6-59 months
* Non-pregnant Women 15-49 years

The Phlebotomist will be responsible for MRDR dosing and specimen collection. Only 4 children 6-59 months and 3 non-pregnant women 15-49 years in each cluster (~720 total children and ~540 total women) will participate in the MRDR dosing substudy.

* Children and women participating in the MRDR dosing substudy will be identified by the team supervisor.
  + Seven doses of vitamin A2 (4 for children 6-59 months and 3 for non-pregnant women 15-49 years) will be prepared (using positive displacement pipettes and tips in the laboratory) prior to going into the field for the day.
  + Doses should be kept cool in the cold box until they are administered to each child.
  + 1mL syringes and spoons will be made available to aid in oral administration of the MRDR dose.
* Using the guidelines provided by CDC, administer the right dose of vitamin A2 to the participants. Give the dose with a teaspoon of vegetable oil (containing no vitamin A) to help get the dose to the stomach and absorbed.
* Fasting conditions are preferred but not required. If the participant has eaten a food that is a good source of vitamin A within the last 2 hours then it will be necessary to wait 2 hours before administering the dose.
* Participants should also be instructed not to consume rich sources of vitamin A during the 4 hours after administering the dose of vitamin A2 and before the second venous blood draw.
* The Phlebotomist will need to return to the participant’s home to collect venous blood using a new **Purple Top Vacutainer** 4 hours after the dosing.
* Once blood has been collected, the Phlebotomist needs to label the Purple Top Vacutainer with the “**MRDR Purple Top**” label and properly store it in a vacutainer rack inside a cold box with frozen gel packs until it is processed.
  + Be sure to keep the MRDR Purple Top label when transferring the remaining labels during the day to the Laboratory Technician with other specimens.
* Find the appropriate participant’s number on the “**Specimen Control Form A**” (**Annex 3a**) and mark collected for MRDR specimens.
* The MRDR specimens will then be transferred by the Phlebotomist to the Laboratory Technician. The Laboratory Technician will have the remaining labels that were transferred previously during the day with other specimens.
* The Phlebotomist should complete the “**Specimen Transfer Form**” (**Annex 3d**) to include the sample IDs and time of blood collection for the MRDR samples. This form will be given to the Laboratory Technician when specimens are transferred.

# Procedures for Specimen Processing in the Field

Blood and urine specimens will be processed by the Laboratory Technician and stool specimens will be processed by the Pathologist.

**Processing Blood and Urine Specimens- Laboratory Technician**

***Duties Prior to Specimen Processing:***

* The Laboratory Technician should pack the backpack with all the supplies needed for daily specimen processing in the field (**Annex 4b**).
* Set up a clean working area to process specimens.
  + Set up all the equipment and supplies needed for specimen processing and storing. This requires a comfortable location with a flat surface sufficiently large to lay out the absorbent pad and all equipment and supplies prior to processing and storing the specimens in the field.
* The Laboratory Technician is responsible for retrieving all specimens from the Phlebotomist and processing the blood specimens in the field. The Laboratory Technician will transfer all stool specimens to the Pathologist along with the appropriate labels for processing.
* The Laboratory Technician must receive a “**Specimen Transfer Form**” (**Annex 3d**) from the Phlebotomist when specimens are transferred.
* The serum from the “**Blue Top Vac**” will be used to measure serum zinc, serum vitamin B12, and ELISA for children 6-59 months and non-pregnant women 15-49 years.
* The “**Red Top Vac**” will be used to for the ELISA for adolescent girls 10-19 years, adolescent boys 10-19 years, and pregnant women 15-49 years.
* Backups of serum from the Blue Top and Red Top Vacutainers will be made when enough serum is available.
* Whole blood lysate will be prepared from the “**Purple Top Vac**” from children 6-59 months, non-pregnant women 15-49 years and adolescent girls 10-19 years.
* The plasma from the “**Purple Top Vac**” will be stored as back-up.
* The urine cups will be used to measure urinary iodine for children 6-59 months, children 6-9 years, and women 15-49 years.
* Plasma from the “**MRDR Purple Top**” will be used for MRDR/retinol.
* Processed specimens will be placed into cryovials and 0.2mL PCR tubes (**Figure 6**).
* The “**BD Purple Top**” will not be processed and should be transported/shipped to Kathmandu for CBC and assessment of blood disorders.
* For each study participant, place the corresponding participant label onto the “**Specimen Control Form B**” (**Annex 3b**) and complete the sections for specimens collected.

**Figure 6. Cryovials and 0.2mL PCR Tubes Used to Store Processing Specimens**

Cryovial

0.2mL PCR Tube

***Processing Blue Top Vacutainers in the Field:***

* + Blue Top Vacutainers will be collected only from children 6-59 months and non-pregnant women 15-49 years.
    - Children 6-59 months and non-pregnant women 15-49 years will have 2 cryovials and one 0.2mL PCR tube for serum:
      1. **0.2mL PCR tube**- for ELISA
      2. **Serum Vial 1**- for zinc and B12
      3. **Serum Vial 2**- backup
  + Centrifuge the Blue Top Vacutainers on medium to high speed for 10 minutes.
    - Transfer 100µl serum into 1- 0.2mL PCR tube and label with the correct participant’s number.
    - Using a disposable transfer pipette, transfer 1mL of serum to a cryovial and affix the “**Serum Vial 1**” label with the correct participant’s number (the number that matches the number from the Blue Top Vacutainer).
    - Transfer the remaining serum into a second cryovial and affix the “**Serum Vial 2**” label with the correct participant’s number.
  + The cryovials and 0.2mL PCR tubes will go into different cryovial boxes according to how they are labelled, as well as separated according to age group (e.g., Serum Vial 1- children 6-59 months, Serum Vial 2- children 6-59 months, 0.2mL PCR tubes- children 6-59 months, etc.).
  + Store the processed specimens in the portable freezer until they are transferred to the laboratory at the end of each day.

***Processing Purple Top and Red Top Vacutainers in the Field:***

The following procedures are performed to process the Purple Top Vacutainers (“**Purple Top Vac**” for back-up plasma and “**MRDR Purple Top**” for MRDR) and Red Top Vacutainers (for ELISA and back-up serum).

* “**Purple Top Vacs**” will be collected for all age groups, except children 6-9 years.
* “**Red Top Vacs**” will be collected from pregnant women 15-49 years, adolescent girls 10-19 years, and adolescent boys 10-19 years.
* “**MRDR Purple Tops**” will be collected only from children 6-59 months and non-pregnant women 15-49 years.
* Store all vacutainers in a cold box with frozen gel packs.

1. **FIRST:** Prepare whole blood lysate (2 vials) for RBC folate analysis from the Purple Top Vacutainers.
2. Centrifuge all “**Purple Top Vacs**” to collect plasma for backup (1 vial).
3. Centrifuge all “**Red Top Vacs**” to collect serum for ELISA and backup (1 vial and 1 PCR tube).
4. Centrifuge the “**MRDR Purple Tops**” to collect the plasma for MRDR/retinol (2 vials).
5. ***Prepare whole blood lysate (2 vials) for RBC folate analysis:***

**Prior to centrifugation of the Purple Top VAC, prepare the whole blood lysate.**

* Whole blood lysate will be prepared for the following age groups:
  + Children 6-59 months
  + Adolescent girls 10-19 years
  + Non-pregnant Women 15-49 years
* Prepare a 1% solution of ascorbic acid in water in a 50mL tube.
  + This solution will need to be prepared fresh daily.
  + Obtain a new vial of ascorbic acid (0.5g).
    - The pre-weighted vials of ascorbic acid should be prepared ahead of time by the Laboratory Technicians and Laboratory Coordinators prior to going into the field.
      * A scientific balance, bottles of ascorbic acid, spatulas, and vials will be provided to pre-weight the ascorbic acid.
  + Remove the cap of the 50mL tube and add bottled water to the 50mL mark.
  + Before opening the lid to the vial containing the ascorbic acid, tap the top of the vial to be sure all powder is at the bottom of the vial.
  + Remove the cap of the 50mL tube and add bottled water to the 50mL tube up to the 50mL mark.
  + Gently open the vial containing the ascorbic acid and pour the powder into the 50mL tube containing the water.
    - Be sure to tap the vial so that all powder is removed.
  + Replace the cap of the 50mL tube and mix until all ascorbic acid is dissolved.
  + Be sure to store the 50mL tube in a cold box with frozen gel packs while not in use.
    - Try to avoid direct contact with sunlight when using the 1% solution of ascorbic acid.
* Pipette 1mL of this 1% solution of ascorbic acid in water into 2 cryovials.
* Mix the “**Purple Top Vac**” well, and then pipette 100µL whole blood into each of the 2 cryovials.
* Cap the cryovials and mix well. Label each with its corresponding “**RBC Folate Vial 1**” and “**RBC Folate Vial 2**” labels.
* Place all cryovials into a separate cryovial box and separate according to age group (e.g., RBC Folate Vial 1- children 6-59 months, RBC Folate Vial 2- children 6-59 months, RBC Folate Vial 1- Adolescent Girls 10-14 years, etc.) and store in the portable freezer.
* Remember, RBC Folate Vials will be prepared only for children 6-59 years, non-pregnant women 15-49 years, and adolescent girls 10-19 years.

1. ***Centrifugation of Purple Top Vacutainers to collect the plasma for backup (1 vial):***

* When time permits in the field, centrifuge the “**Purple Top Vac**”.
* Centrifuge the specimens at medium to high speed for 10 minutes.
* Transfer the plasma into a cryovial and label with the corresponding participant’s “**Plasma Vial 1**” label.
* Place all “**Plasma Vial 1”** vials into a separate cryovial box according to age group (e.g., Plasma Vial 1- children 6-59 months, Plasma Vial 1- non-pregnant women 15-49 years, etc.) and store in the portable freezer.

1. ***Centrifugation of Red Top Vacutainers to collect serum for ELISA (1 PCR tube) and backup (1 vial- Serum Vial 2):***

* **“Red Top Vac”** vacutainers will be collected from the following age groups:
  + Pregnant women 15-49 years
  + Adolescent girls 10-19 years
  + Adolescent boys 10-19 years
* When time permits in the field, centrifuge the “**Red Top Vac**” Vacutainers at medium to high speed for 10 minutes.
  + - Using a disposable transfer pipette, Transfer 100µl serum into 1- 0.2mL PCR tube and label with the correct participant’s number.
    - Transfer the remaining serum into a cryovial and affix the “**Serum Vial 2**” label with the correct participant’s number.
      * **NOTE**: Red Top Vacutainers will not be aliquoted into a Serum Vial 1 because these participant samples will not be shipped for serum zinc and serum B12 analysis.
  + The cryovials and 0.2mL PCR tubes will go into different cryovial boxes according to how they are labelled, as well as separated according to age group (e.g., Serum Vial 1- pregnant women 15-49 years, Serum Vial 2- pregnant women 15-49 years, 0.2mL PCR tubes- pregnant women 15-49 years, etc.).
  + Store the processed specimens in the portable freezer until they are transferred to the laboratory at the end of each day.

1. ***Centrifugation of MRDR Purple Top Vacutainers to collect the plasma for MRDR/retinol (2 vials):***

* **“MRDR Purple Top”** Vacutainers will be collected from the following age groups:
  + Children 6-59 months
  + Non-pregnant Women 15-49 years
* When time permits in the field, centrifuge the “**MRDR Purple Top**” Vacutainers.
* Centrifuge the “MRDR Purple Tops” at medium to high speed for 10 minutes.
* Using a disposable transfer pipette, pipette 1mL plasma into two cryovials.
* Cap the cryovials well. Label each with its corresponding “**MRDR Vial 1**” and “**MRDR Vial 2**” labels.
* Place all cryovials into a separate cryovial box and separate according to age group (e.g., MRDR Vial 1- children 6-59 months, MDRD Vial 2- children 6-59 months, MRDR Vial 1- Non-pregnant women 15-49 yrs, etc.) and store in the portable freezer.

***Processing Urine Specimens in the Field:***

* Urine will be collected from the following age groups:
  + Children 6-9 years
  + Women 15-49 years (Pregnant and Non-Pregnant)
* The Laboratory Technician will divide the urine into cryovials.
* Pre-label 2 cryovials with the pre-printed labels for each participant.
* One cryovial should be a 5mL vial and one cryovial should be a 2mL cryovial for back-up.
* Gently mix the urine before aliquoting (be sure the cap of the urine cup is securely tightened).
* Using a disposable transfer pipette, transfer ~4mL urine into the 5mL cryovial and ~1.5mL urine into the 2mL cryovial and cap.
* Place the cryovials into 2 labelled cryovial boxes (one for Urine Vial 1 and one for Urine Vial 2).
* Find the appropriate participant’s number on the “**Specimen Control Form** **A**” (**Annex 3b**) and mark collected for urine.
* Place all cryovials into a separate cryovial box and separate according to age group (e.g., Urine Vial 1- children 6-9 years, Urine Vial 2- children 6-9 years, etc.) and store in the portable freezer.

**Processing Stool Specimens- Pathologist**

***Duties Prior to Specimen Processing:***

* The Pathologist should pack the backpack with all the supplies needed for daily specimen processing in the field (**Annex 4c**).
* Set up all the equipment and supplies needed for specimen processing and storing. This requires a comfortable location with a flat surface sufficiently large to lay out the absorbent pad and all equipment and supplies prior to processing and storing the specimens in the field.
* The Pathologist is responsible for retrieving all stool specimens from the Laboratory Technician once they are delivered by the Phlebotomist.
* One stool cup will be received for each participant
  + One aliquot of the stool (~1g) will be used for the Kato Katz method in the field.
  + A second aliquot (~1g) of each specimen will be transferred to cryovial and labelled “**Stool Vial 1**” for *H. pylori* analysis.
* For each participant, place the corresponding label onto the “**Specimen Control Form C**” (**Annex 3c**) and complete the sections for specimen processing.

1. ***Prepare cryovial for H. pylori analysis:***

* Using a disposable wooden spatula, transfer ~1g of stool into one cryovial and cap.
* Label each cryovial with its corresponding “**Stool Vial 1**” label.
* Place all labelled “**Stool Vial 1**” specimens a cryovial box labelled “Stool Vial 1” and place into the portable freezer to be transferred to the central laboratory at the end of each day.
* Complete the appropriate section for specimen processing on the “**Specimen Control Form** **C**” (**Annex 3c**).

1. ***Kato Katz preparation and analysis:***

* The Kato Katz procedure should be performed on each stool specimen (**Annex 22: Kato Katz Procedure**).
  + ~1g of stool is needed for Kato Katz preparation and analysis.
  + Label 2 prepared microscope slides with its corresponding “**Kato Katz Slide**” labels.
  + Once the microscope slides have been prepared and read, be sure to record the results on the “**Specimen Control Form C**” (**Annex 3c**).
  + Place all labelled “**Kato Katz Slides**” into a slide box to be stored until transported to the central public health laboratory.

# Sorting Cryovials into Cryovial Boxes

Cryovials must be sorted properly into cryovial boxes according to specimen type and age group. This is essential to ensure the correct specimens are shipped to the correct laboratory for processing. Laboratory Technicians are responsible for sorting the cryovials into the correct cryovial boxes according to specimen type and age group during the processing of specimens. Laboratory Coordinators are responsible for ensuring that the Laboratory Technicians have properly performed this task.

* Once all specimens for 1 participant have been processed, begin to separate the specimens into separate cryovial boxes.
  + For example, all cryovials labelled “Serum Vial 1” and belong to children 6-59 months will be placed into 1 box labelled Serum Vial 1- children 6-59 months, all specimens labelled “Serum Vial 2” will be placed into 1 box labelled Serum Vial 2- children 6-59 months, etc.
  + Serum Vial 1 (1 from children 6-59 months and 1 from non-pregnant women 15-49 years), 0.2mL PCR Tubes, RBC Folate Vial 1, and MRDR Vial 1 will be shipped to laboratories for analysis.
  + Serum Vial 2, Plasma Vial 1, RBC Folate Vial 2, and MRDR Vial 2 will serve as back-up specimens and will be stored long-term at -70°C.
  + The Laboratory Coordinators will assign each cryovial box will get a unique number as well as inventory the specimens in each box to be given to each laboratory for analysis (See labelling of cryovial boxes).

# Transportation of Specimens from the Field to the Laboratory

***At the end of the day:***

The Laboratory Coordinator, with the assistance of the Laboratory Technician, is responsible for ensuring that all the blood specimens are consolidated and transferred to the laboratory for storage.

The Laboratory Coordinator, with the assistance of the Laboratory Technician, is responsible for ensuring all ““**BD Purple Top**” blood specimens are consolidated and transferred to Kathmandu for analysis. It is important that these specimens are shipped using frozen gel packs protected by bubble wrap. **THESE SPECIMENS ARE NOT TO BE FROZEN. THEY ARE TO BE SHIPPED COLD.**

* All of the gel packs used in the field should be frozen until they are hard every night.
* The freezer must be set to at least -20º C.
* **It is critical the gel packs are completely frozen until hard before teams leave for the field each day.**

# Lab Procedures for Specimen Storage

***Storing Specimens in the Central Public Health Center Laboratories (Districts):***

* All specimens need to be stored properly in a -20°C freezer within each district until they are transferred to the National Public Health Laboratory (NPHL) in Kathmandu.
* The Laboratory Coordinator should ensure that all cryovial boxes have been accurately labelled with the specimen type and age group.
* All specimens will be transferred to the NPHL in Kathmandu at the end of the survey.

***Storing Specimens in the National Public Health Laboratory (Kathmandu):***

* All specimens need to be stored properly in a -86°C freezer until they are shipped for analysis.
* Cryovial boxes will then be numbered numerically so that the specimen inside the box can be assigned to a specific cryovial box for the specimen inventory.
  + Box numbers will be assigned once all specimens are received at NPHL.
* A specimen inventory should be created for all specimen boxes and divided according to specimen type.
* All backup specimens will be stored long-term in the laboratory preferably at -86°C.

# Specimen Shipment

Supplies Needed:

* Styrofoam cold boxes
* Bubble wrap
* Dry ice (1 pound for every 2 hours of shipping) or frozen gel packs
* Packing tape
* Dry ice labels (if dry ice is used)
* Cryovial boxes with specimens packed for shipment
* Copy of specimen inventory for the specimen type being shipped (a copy of the

inventory should also be emailed to the laboratory)

The Laboratory Coordinators are responsible for shipping specimens to the correct laboratory for analysis (see **Table 2** below).

**Table 2. Laboratories Identified to Analyze Survey Specimens**

|  |  |  |
| --- | --- | --- |
| Laboratory | Contact | Specimens Shipped |
| Germany | Juergen Erhardt  Vit  Kastanienweg 5,77731 Willstaett, Germany  Tel: +49-7852-1805  Email: [erhardtj@gmail.com](mailto:erhardtj@gmail.com) | 0.2mL PCR Tubes |
| INCAP | Dora Inés Mazariegos Cordero  Unidad de Nutrición y Micronutrientes (UT-UB)  Instituto de Nutrición de Centroamérica y Panamá (INCAP)  Calzada Roosevelt 6-25 zona 11, Apartado Postal 1188,  Guatemala, Centro América  Tel: (502) 2472-3762 ext 1212  Fax: (502) 2473-6529  Email: [dmazariegos@incap.int](mailto:dmazariegos@incap.int) | MRDR Vial 1, Urine Vial 1, Serum Vial 1 |
| JUST Laboratory | Ibrahim M.D. Khatib (MSc, PhD) Associate Prof. - Metabolic & Community Nutrition, Dept of Community Medicine -Faculty of Medicine Jordan University of Science & Technology, (JUST), Irbid  21110, (POB 2001) Jordan. Mobile phone: +962 - 79 - 5668655 Phone :  0962 - 2 - 7100870 ; Fax : 0962-2-7201064 Email : [khatibmd@orange.jo](mailto:khatibmd@orange.jo) | Serum Vial 1 |
| Peking University | Jianmeng Liu, MD, PhD  Professor, Deputy Director  Institute of Reproductive and Child Health  Peking University  38 College Road  Haidian District  Beijing China  Tel: 86-10-82801136  Email: [liujm@pku.edu.cn](mailto:liujm@pku.edu.cn) | RBC Folate Vial 1 |
| NPHL  and  Saymak Diagnostics | Dr. Geeta Shakya  National Public Health Laboratory Teku, Kathmandu, Nepal Phone : 977-1-4252421, 4240217 Fax : 977 – 4252375  [geeta.nphl@gmail.com](mailto:geeta.nphl@gmail.com)  [nphl@wlink.com.np](mailto:nphl@wlink.com.np)  Dr. Keyoor Gautam  Samyak Diagnostic  2nd Floor Norkhang Complex  Lalipur Nepal  Phone: 977 - 5009477  [drkeyoor@gmail.com](mailto:drkeyoor@gmail.com) | BD Purple Top Vacutainers  NPHL for CBC and Saymak Diagnostic for Blood Disorders |
| WARUN/AFRIMS | Dr. Sanjaya Kumar Shrestha  Walter Reed/Armed Forces Research Institute of Medical Sciences Research Unit Nepal  email: [ShresthaSK.ca@afrims.org](mailto:ShresthaSK.ca@afrims.org)  Phone: 977 - 9802042523 | Stool Vial 1 |

1. Specimens will be stored in a -86°C freezer until they are shipped.
2. Be sure to ship the correct specimens to the correct laboratory
   1. Cryovial boxes containing 0.2mL PCR tubes will be shipped to Juergen Erhardt in Germany.
   2. Cryovial boxes containing “**RBC Folate Vial 1**” vials will be shipped to the Institute of Reproductive and Child Health, Peking University in Beijing, China.
   3. Cryovial boxes containing “**Serum Vial 1**”, “**Urine Vial 1**”, and “**MRDR Vial 1**” vials will be shipped to INCAP in Guatemala.
   4. Cryovial boxes containing “**Stool Vial 1**” vials will be transferred to AFRIMS in Kathmandu.
   5. All “**BD Purple Tops**” will be transferred to Kathmandu for CBC analysis at NPHL and assessment of blood disorders at Samyak Diagnostic.
   6. All backup specimens (vials labelled **Serum Vial 2, Plasma Vial 1, RBC Folate Vial 2**, **Urine Vial 2**, and **MRDR Vial 2**) will remain in the laboratory and stored preferably at -70°C.
3. Assemble all materials for packing and shipping frozen specimens including Styrofoam cold box, dry ice/frozen gel packs, bubble wrap, Cryo-Temp monitor, dry ice labels and packing tape.
4. Places the dry ice at the bottom of the Styrofoam cold box. If there is no dry ice, place 6-8 gel packs at the bottom of the box and follow the next steps.
5. Place a layer of bubble wrap over the dry ice/gel packs so that the specimens don’t touch the dry ice/gel packs.
6. Wrap the plastic storage boxes with bubble wrap and place the boxes inside the Styrofoam cold box.
7. Fill empty space on top and sides of the Styrofoam cold box with dry ice/gel packs. Close the lid of the box.
8. Secure the outer lid of the box with tape. When using dry ice, the packaging must permit release of carbon dioxide gas to prevent a build-up of pressure that could rupture the package (DO NOT tape all around box).
9. Record the date and time the specimens are being transported from the Nepal to the designated laboratory conducting the analysis on the “**Specimen Control Form B**” (**Annex 3b**). Make a photocopy of these forms and keep the original at the national laboratory in Nepal. Place the photocopies in a sealed Ziploc bag and include in the box for shipment.
10. Label the shipping box with a dry ice sticker (if dry ice is used) and a keep frozen

sticker.

1. Label each shipping box with the address of the recipient lab. Include name, telephone number, and return address information. Contact the recipient lab before shipping the specimens to ensure that the staff is ready to receive the shipment and contact the recipient after shipment to confirm the delivery of specimens.

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**Annex 1: Universal Precautions**

1. Universal precautions are defined by CDC as a set of precautions designed to prevent transmission of human immunodeficiency virus (HIV), Hepatitis B virus (HBV), and other blood-borne pathogens.
2. Blood and other patients’ body fluids are considered potentially infectious for HIV, HBV, and other blood-borne pathogens.
3. Therefore health-care workers who handle body fluids such as blood, mucus, sputum, urine, stool, etc. should observe the following precautions:

* Prevent skin and mucous-membrane exposure when handling blood or other blood-borne pathogens.
* Use personal protection barriers (e.g. gloves, lab coats and eye glasses).
* Wash hands after removing the gloves.
* Clean laboratory benches before and after procedures with an appropriate disinfectant.
* Dispose needles in sharps containers to prevent injuries.
* Dispose cuvettes and all other used materials in biohazard bags for incineration or appropriate disposal.
* Immediately report all accidents or injuries to your supervisor and follow the below precautionary measures:
  + In case of injury, it is necessary to squeeze the blood out of the injury, thoroughly wash the injury with soap and running water, cleanse the skin with 70% alcohol.
  + In case of contamination of hands with the blood, immediately wash the hands with warm water and soap.
  + In case blood gets to face, it should be thoroughly washed with warm water and soap.
  + Test the specimen of the source individual for HIV and hepatitis as early as possible (within 24 hours of exposure).
  + Document the following data, related to the nature of exposed, status of source individual & status of exposed health worker
* Name and data of the source individual.
* Time & date of exposure.
* Nature of exposure.
* Body site exposed.
* Infective status of the source.
* Previous testing & Immune status of the exposed health worker.
* Seek medical assistance as soon as possible

**Annex 2: Cold Chain Logistics**

|  |  |
| --- | --- |
| Frozen Gel Packs | Fresh frozen gel packs MUST be used at the beginning of each day. The Phlebotomists, Lab Technicians, and Pathologists must store the gel packs at -20º C in the portable freezer when not in use. Whenever possible, gel packs that have thawed while in the field must be replaced with fresh frozen gel packs (i.e., when temperature of the cold box reaches ~6°C call the Supervisor to have a Driver deliver new frozen gel packs). At the end of the day, gel packs used in the field should be placed in the portable freezers and kept at least at -20ºC so that they will be frozen and ready for the next day. A Driver will deliver the portable freezer to the laboratory to be plugged into electricity overnight. The Driver will then transport the portable freezer with frozen gel packs back to the field at the beginning of the following day. |
| Vacutainers, Urine Cups, and Stool Specimens | After blood collection, the Phlebotomists should place the labelled vacutainers, urine cups and stool specimens into the cold box containing frozen gel packs. Bubble wrap should be placed between the specimens and the frozen gel packs so that they do not touch. All vacutainers and urine cups will be processed by the Laboratory Technicians in the field. All the specimens in the cold box and the portable freezer must be sent at the end of the day for processing and storage. Stool specimens are given to the Pathologist for further processing and the BD purple top vacutainers are not processed, but transported to Kathmandu for CBC and blood disorder analysis. |
| Cold Boxes | The Phlebotomists, Lab Technicians, and Pathologists should keep the cold box closed at all times. Avoid leaving the lid open and exposure to direct sun. A digital thermometer will be included in each cold box. The temperature of the cold box should remain <8°C. When temperature of the cold box reaches ~6°C call the Supervisor to have a Driver deliver new frozen gel packs. |
| Blue Top, Red Top, and Purple Top Vacutainers | The blue top, red top, and purple top vacutainers should be processed in the field the same day as collection as time allows. After centrifuging the specimens, the Lab Technician should transfer the serum/plasma into labelled cryovials (or PCR tubes) and stored in the portable freezer until they are transported back to the laboratory at the end of the day. Serum from the blue top vacutainers will be used for ELISA, serum zinc, and serum B12. The purple top vacutainer is used to 1) measure hemoglobin, 2) test for malaria, 3) test for visceral leishmaniasis; 4) prepare RBC lysate for folate, and 5) centrifuged as a backup specimen. Serum from the red top vacutainer will be used for ELISA. |
| BD Purple Top Vacutainer | The BD purple top vacutainers will NOT be processed in the field. The BD purple top vacutainers will be transported to Kathmandu for CBC analysis at NPHL and later transported to another lab for assessment of blood disorders. |
| MRDR Purple Top Vacutainer | The MRDR purple top vacutainer will be used for the MRDR/retinol subset. After centrifuging the blood specimens, the Lab Technician should be transfer 1mL plasma into one cryovial and the remainder into a second cryovial and label each and placed into a -20ºC/-70ºC freezer. Plasma specimens should not be left at room temperature for more than 1 hour. Frozen plasma specimens should NOT be left at room temperature for more than 15 minutes to avoid thawing the specimens. |
| Urine Cup | Urine specimens should be processed by the Laboratory Technician in the field as time permits. The urine cup should be mixed well and then two aliquots made: 1 aliquot of ~4 mL urine aliquoted into a 5 mL cryovial and 1 aliquot of ~1.5mL into a 2 mL cryovial for backup, labelled, and placed into a -20ºC/-70ºC freezer. |
| Stool Specimens | The Pathologist will process all stool specimens. Approximately 1g of stool will be used to assess STHs in the field using the Kato Katz method. A second aliquot of 1g of stool should be placed into 2mL cryovial for transport to Kathmandu for assessment of *H. pylori*. |

**Annex 3a: Specimen Control Form A-Phlebotomists**





**Annex 3b: Specimen Control Form B-Laboratory Technicians**







**Annex 3c: Specimen Control Form C-Pathologists**





**Annex 3d: Specimen Transfer Form- Phlebotomists to Lab Technicians**



**Annex 4a: List of Supplies Needed Daily by Phlebotomists**

|  |  |
| --- | --- |
| **List of Supplies Needed Daily by Each Phlebotomist**  \*\*Includes enough supplies for 4 households (plus extra supplies) | |
| Supply Item | Quantity Required |
| Backpack | 1 |
| Blue Top Vacutainers | 10 |
| Purple Top Vacutainers | 20 |
| Red Top Vacutainers | 12 |
| Tourniquet | 2 (can be reused) |
| 23G Needles | 16 |
| 21G Needles | 10 |
| Vacutainer Barrels | 2 (can be reused) |
| Sharps container | 1 Box |
| Alcohol pads | 20 |
| Gauze pads | 20 |
| Absorbent pads | 2 (can be reused if clean) |
| Biohazard bags | 2 |
| Ziploc bags | 1 Large (for the Specimen Control Form) and 1 Small (for Cryovial labels) |
| Labels | 4 sets pre-printed (will be transferred from Enumerator) for each age group  9-12 additional blank labels (ONLY for emergencies) |
| Cold box | 1 |
| Frozen Gel Packs | 4-5 for cold box |
| Bubble Wrap | 1 Small Piece (to protect specimens from frozen gel packs inside cold box) |
| Digital Thermometer | 1 |
| HemoCue 301 | 1 |
| HemoCue 301 Cuvettes | 1 container (with a minimum of 20 cuvettes) |
| Batteries | 4 (Extra for HemoCue 301) |
| Vacutainer Rack for Cold Box | 1 |
| Cryovial Box | 1 (used to hold vacutainers during specimen collection) |
| MRDR Doses + syringes | 7 doses (syringes will aid in oral administration of the dose) |
| Kimwipes | 1 Box |
| Band-aids | 20 |
| Gloves S, M, L | 1 Box of correct size (with a minimum of 20 pairs) |
| Malaria Test Kits & Buffer | 20 Kits (1 bottle of butter) |
| V. Leishmaniasis test Kits | 20 Kits (1 bottle of butter) |
| H. pylori test kits | 10 Kits (1 bottle of butter) |
| Urine/stool collection cups | 20 |
| Timers | 2 |
| Clip Board | 1 |
| Log Forms | Specimen Control Form A (1), Referral Slips (20 each for Malaria, Anemia, and VL), Referral Logs (2 each for Malaria, Anemia, VL, and H pylori), and Specimen Transfer Forms (4) |
| Pens | 2 |
|  |  |

**Annex 4b: List of Supplies Needed Daily by Laboratory Technicians**

|  |  |
| --- | --- |
| **List of Supplies Needed Daily by Laboratory Technician**  \*\*Includes enough supplies for 12 households (plus extra supplies) | |
| Supply Item | Quantity Required |
| Backpack | 1 |
| Absorbent pads | 4 |
| Biohazard bags | 4 |
| Ziploc bags | 1 Large (for the Specimen Control Form) and 1 Medium (for PCR Tubes) |
| Labels | 12 sets pre-printed (will be transferred from Phlebotomist) for each age group  9-12 additional blank labels (ONLY for emergencies) |
| Cold box | 1 |
| Portable Freezer | 1 |
| Frozen Gel Packs | 28 Frozen Gel Packs (4 for Cold Box and 24 for Portable Freezer |
| Bubble Wrap | 1 Small Piece (to protect specimens from frozen gel packs inside cold box) |
| Digital Thermometer | 1 |
| Vacutainer Rack | 1 |
| Cryovial Box | 12 |
| Cyrovials | 120 |
| PCR Tubes | 20 |
| 50mL Tube | 1 Tube |
| Vial containing Ascorbic Acid (0.5g) | 1 Vial |
| Bottled Water | 1 Bottle (for 1% ascorbic acid solution) |
| Pipettes | 2 (1 of each volume/type) |
| Pipette tips | 2 boxes (1 of each volume) |
| Disposable Transfer Pipettes | 60 |
| Kimwipes | 1 Box |
| Gloves S, M, L | 1 Box of correct size (with a minimum of 30 pairs) |
| Portable Centrifuge | 1 |
| Clip Board | 1 |
| Forms | Specimen Control Form (1) |
| Pens | 2 |

**Annex 4c: List of Supplies Needed Daily by Pathologist**

|  |  |
| --- | --- |
| **List of Supplies Needed Daily by Pathologist**  \*\*Includes enough supplies for 12 households (plus extra supplies) | |
| Supply Item | Quantity Required |
| Backpack | 1 |
| Absorbent pads | 4 |
| Biohazard bags | 4 |
| Ziploc bags | 1 Large (for the Specimen Control Form) and 1 Medium (for PCR Tubes) |
| Labels | 12 sets pre-printed (will be transferred from Phlebotomist) for each age group  9-12 additional blank labels (ONLY for emergencies) |
| Cold box | 1 |
| Portable Freezer | 1 |
| Frozen Gel Packs | 28 Frozen Gel Packs (4 for Cold Box and 24 for Portable Freezer |
| Bubble Wrap | 1 Small Piece (to protect specimens from frozen gel packs inside cold box) |
| Counter | 1 |
| Vacutainer Rack | 1 |
| Microscope slides | 48 |
| Slide Box | 2 |
| Microscope | 1 |
| Kato Katz Supplies | Enough supplies for 24 stool samples |
| Cryovials for H. pylori | 24 |
| Kimwipes | 1 Box |
| Gloves S, M, L | 1 Box of correct size (with a minimum of 30 pairs) |
| Portable Centrifuge | 1 |
| Clip Board | 1 |
| Forms | Specimen Control Form (1) |
| Pens | 2 |

**Annex 5: Flow Chart for Specimen Field Testing and Field Processing**



**Annex 6: Procedure for Venous Blood Collection**

1. Obtain informed consent for blood collection.
2. Set up all the supplies needed for the blood collection. This requires a comfortable location with a flat surface sufficiently large enough to lay out the absorbent pad and all equipment and supplies prior to collecting the venous sample from the participant.
3. For small children, have the mother or caretaker hold the child on her lap comfortably and instruct the mother in how to hold the child to minimize the child’s movement during the venous sample collection.
4. Lay out all blood collection supplies onto a disposable absorbent pad, including vacutainers required for the specific age group blood is being collected from.
5. Assemble needle or butterfly needle into Vacutainer holder being sure that it is firmly seated into threads. Loosely place Vacutainer tube[[1]](#footnote-1) into holder, but do *not* puncture top. Assemble and open supplies needed for collection.
6. Examine both arms to find the best vein. Locate the puncture site. Apply tourniquet (not too tightly).
7. Wipe the area with an alcohol swab in a circular motion making sure the area is thoroughly clean. Dry with gauze.

1. If it is necessary to feel the vein again, do so. After you feel it, cleanse the area again with an alcohol swab. Dry with gauze.
2. Fix the vein by pressing down on the vein about 1 inch below the proposed point of entry into the skin and pull the skin taut.
3. Remove needle shield.
4. Approach the vein in the same direction the vein is running. Hold the needle so that it is at an approximately 15° angle with the participant's arm.
5. Push the needle with bevel facing up firmly and deliberately into the vein. Activate the vacuum collection tube by pushing the tube onto the needle and puncturing the tube top. If the needle is in the vein, blood will flow freely into the tube. If no blood enters the tube, probe once or twice for the vein until entry is indicated by blood flowing into the tube.
   1. The Phlebotomist will be given only two chances to collect blood from each participant.

**Annex 7: Procedure for checking Malaria using Rapid Malaria Antigen HRP2/pLDH Combo Test Kit**

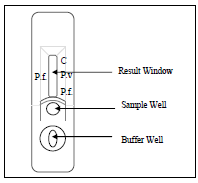
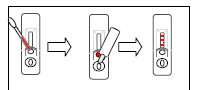
Malaria is a serious, sometimes fatal parasitic disease characterized by fever, chills and anemia and is caused by a parasite that is transmitted from one human to another by the bite of infected Anopheles mosquitoes. Four kinds of malaria species that can infect humans: *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*.

The rapid malaria antigen (HRP2/pLDH) combo test kit for *Plasmodium falciparum* and *P. vivax*. The test contains a membrane pre-coated with two monoclonal antibodies as two separate lines across the test strip: one monoclonal antibody for *P. falciparum* and one monoclonal antibody for *P. vivax*.

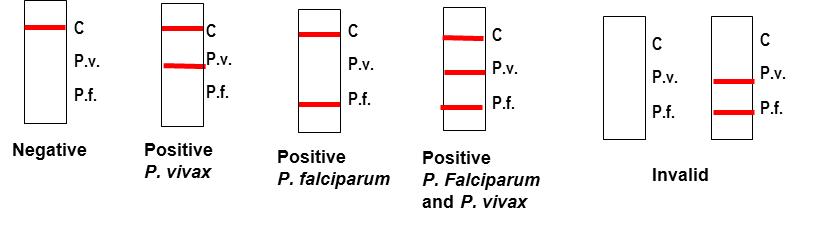
***Procedure:***

1. Set the timer to 20 minutes (do not start the timer at this point).
2. Lay out all items needed for testing malaria, including specimen pipette, assay buffer, test card, and purple top vacutainer containing blood.
3. Carefully remove the cap from the vacutainer and using the specimen pipette provided immerse it into the vacutainer containing blood and collect 5µL of blood. Gently release the pressure on the bulb of the specimen pipette to draw blood into the specimen pipette up to pipette guideline. **DO NO COLLECT TOO MUCH BLOOD FROM THE VACUTAINER**.
4. Add the 5 μL of blood into the “Sample Well” by squeezing the pipette.
5. Add 2 drops (60 μL) of assay buffer into the “Buffer Well”.
6. Start the timer, wait for 20 minutes and read the test result.

**Test Procedure**

**Interpretation of the Test**



1. Positive reaction: The presence of three color bands indicates a positive result for *P. falciparum* and *P. vivax*. The presence of two color bands at C and *P.v* or C and *P.f* indicates a positive test for *P. vivax* or *P. falciparum*, respectively.
2. Negative reaction: The presence of only one band within the result window indicates a negative result.
3. Invalid: The test is invalid if the C line does not appear. The test is also invalid if no lines appear. If either of these occurs, the test should be repeated using a new test.

**Annex 8: Malaria Status and Referral Slip**



**Annex 9: Referral Log for Participants Who Tested Positive for Malaria**



**Annex 10: Procedure for Checking Visceral Leishmaniasis Using the IT LEISH Rapid Test Kit**

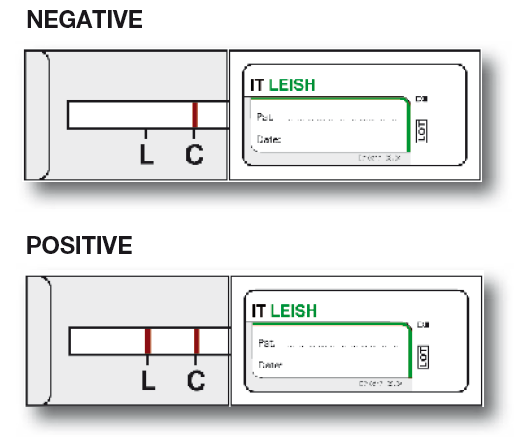
Leishmaniasis is a vector-borne disease transmitted by phlebotomine sandflies in many tropical and sub-tropical countries. Visceral leishmaniasis (VL) is the most series form of the disease, and has an estimated incidence of 500,000 new case and 60,000 deaths per year.

VL diagnosis tests used in the field are based on anti-Leishmania antibodies detection. The BIO-RAD rapid test kit (RTK) will be among the most widely used because it is based on the rK39 protein.

***Procedure:***

1. Set the timer for 20 minutes.
2. Lay out all items needed for testing VL, including specimen pipette, assay buffer, test card, and purple top vacutainer containing blood.
3. Carefully remove the cap from the vacutainer and using the specimen pipette provided immerse it into the vacutainer containing blood and collect 5µL of blood. Gently release the pressure on the bulb of the specimen pipette to draw blood into the specimen pipette up to pipette guideline. **DO NO COLLECT TOO MUCH BLOOD FROM THE VACUTAINER**.
4. Add the 5 μL of blood into the “Sample Well” by squeezing the pipette.
5. Add 2 drops (60 μL) of assay buffer into the “Buffer Well”.
6. Start the timer, wait for 20 minutes and read the test result.

**Test Procedure**



**Annex 11: Visceral Leishmaniasis Status and Referral Slip**



**Annex 12: Referral Log for Participants Who Tested Positive for Visceral Leishmaniasis**

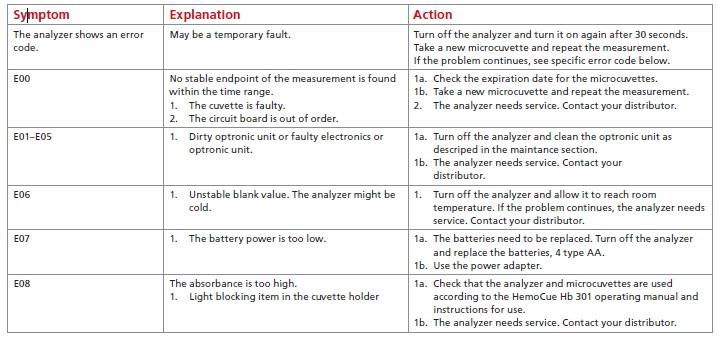


**Annex 13: Procedure for Maintenance of HemoCue and Measuring Hemoglobin Using the HemoCue 301-Photometer**

HemoCue Hb-301 is used to check the hemoglobin (Hb) concentration in the blood. This is a robust instrument that can give accurate readings in a field setting. However, errors in Hb assessment occur if appropriate procedures and techniques are not followed. Use of inappropriate procedures/techniques may cause wide variations in Hb values leading to erroneous estimates of anaemia prevalence in the population.

1. **HemoCue 301 Maintenance:**
2. At the beginning of each survey day, ensure that the instrument is clean (i.e., free of blood and dirt), operational (i.e., turn on the instrument to test for any errors) and contains batteries.
3. If readings are in question, clean the cuvette holder with a dry KimWipe and dry completely before measuring the hemoglobin.
4. If readings continue to be outside the correct range (<4 g/dL or >18 g/dL) or reads ERROR, do not use the instrument. It should be serviced or replaced.
5. Perform daily QC by measuring and recording the results for each of the low, normal and high range control vials on the “HemoCue® Hemoglobin Quality Control Form” (**Annex 16**).
   * This is to be done in the morning (before blood collection begins) and in the evening (when blood collection is finished for the day).
   * Be sure to store QCs in a refrigerator (1-6°C).
6. **Measuring Hemoglobin using HemoCue 301:**
7. Turn ON the HemoCue Hb-301 photometer. **As this instrument has self-test, it does not have a control cuvette and does not need any liquid controls.**
8. In about 30 seconds three lines show up the photometer screen (- - -).
9. Collect blood from the Purple Top Vacutainer using a cuvette
   1. Remove the cap from the Purple Top Vacutainer and gently tilt the vacutainer so that the cuvette can be inserted into the mouth of the vacutainer to fill the cuvette.
10. Clean any excess blood from the cuvette using a Kimwipe or a tissue paper. Do not touch the open end of the cuvette with the wipe. Inspect the cuvette for air bubbles, and if any air bubbles are seen, discard the cuvette and use a fresh cuvette.
11. Place the cuvette in HemoCue holder and gently close the holder into the photometer. The results will be displayed in approximately 10-20 seconds.
12. Record the hemoglobin results on the “**Hemoglobin Status and Referral Slip**” (**Annex 14**) and give to participant’s mother or caretaker or the participant if s/he is an adult. Explain the result to the participant or mother/caregiver. Dispose of the cuvette in sharps container. Refer participant to the local clinic if hemoglobin level is less than the recommended WHO cut-off for severe anemia.
13. Properly discard all used materials according to the biological waste disposal laws of the country in which the survey is taking place.

***HemoCue Trouble Shooting Guide:***



1. **Common Problems to Avoid:**

The key points to be strictly followed during the use of HemoCue, Hb-301 and capillary sampling procedures:

1) Keep the instrument clean, especially the cuvette holder.

A swab dabbed with alcohol can be used to clean away any dirt or dried blood. This should be done at least once a day or when there is a visible build-up of dirt or blood. Be sure the cuvette holder is dry before re-inserting it in the machine.

2) Keep cuvettes clean, dry and away from heat.

Cuvettes in closed containers are good for 3 months after opening. Always keep the container lid closed when not in use to avoid unnecessary exposure of the cuvettes to air, especially in humid conditions. Heat and moisture will denature the chemicals in the cuvette which can lead to inaccurate Hb measurements.

3) Avoid use of poor techniques.

* 1. DO not touch cuvettes with wet fingers. Avoid removing a cuvette from its container when your fingers are wet with alcohol. Alcohol coming in contact with the cuvette can denature the needed chemical in the cuvette selected, as well as, other cuvettes still in the container.
  2. Adequately fill the cuvette. The cuvette needs to be filled with a drop of blood in one continuous motion. Again this depends on the flow of blood and the size of the drop formed; if it is not adequate, the cuvette will not fill adequately. Do not “top off” the cuvette that is not completely filled. This results in erroneous Hb readings...usually too high. Any sign of air-bubbles means that the cuvette has not been filled adequately and should be discarded and a new cuvette used. The presence of bubbles will usually underestimate the Hb reading.
  3. Do Not “slam” the cuvette holder into position for reading. This will avoid spraying blood droplets into the cuvette holder which can hamper the scanner.

1. **Summary of common problems and solutions related to capillary sampling and use of the HemoCue 301 photometer:**

|  |  |
| --- | --- |
| PROBLEM | SOLUTION |
| Not preparing all needed materials before testing a subject. | Place cuvette and vacutainer on work surface; turn on photometer; pull out the cuvette holder to “locked” position so that digital screen reads “READY”; put on gloves. |
| Selecting a cuvette from its jar with fingers wet with alcohol (the alcohol denatures the chemicals inside the cuvette; thus, the selected cuvette as well others inside the jar can be denatured). | Take cuvette out of its container before handling a wet alcohol swab. |
| Holding cuvette in inverted position (slit facing down) during filling (this can lead to air bubbles being trapped resulting in erroneous result). | Hold the cuvette with the slit facing up and the pointed tip touching the blood drop. |
| “Topping off” a partially filled cuvette with repeated blood collection (the reagents in the cuvette are denatured upon contact with the initial amount of blood; red cells of blood introduced later will not be adequately analyzed). | Allow a large blood drop to form on the heel/finger so that it will completely fill the cuvette in one motion. Once filled, hold the cuvette in place for about 2-3 seconds longer to ensure complete filling. |
| Not cleaning off blood on outside of cuvette before testing (can result in erroneously high Hb reading). | Wipe off excess blood from sides of cuvette using a “butter knife” motion to ensure that blood from inside the cuvette is not removed. |
| “Slamming” the cuvette holder into place (can lead to blood drops spattering inside the reading chamber). | Push the cuvette holder gently into position. Once or twice a day clean the cuvette holder with alcohol swab and completely dry before testing. Periodically clean the reading chamber with dry gauze. |

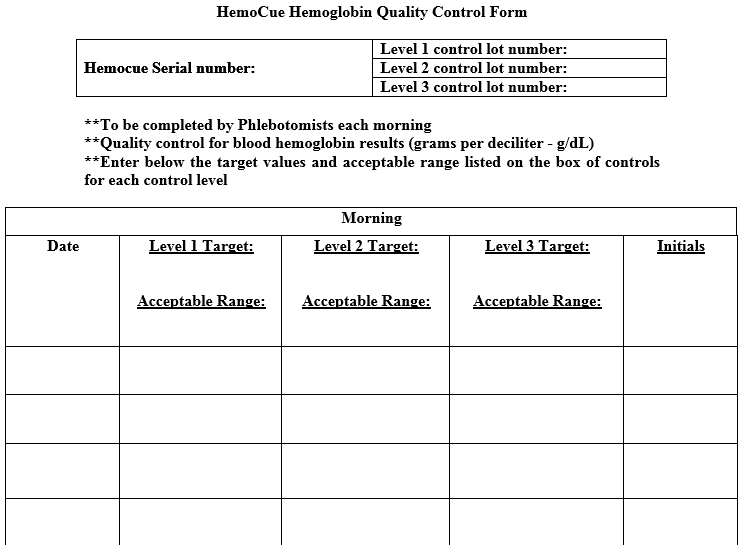
**Annex 14: Hemoglobin Status and Referral Slip**



**Annex 15: Referral Log for Participants Who Are Anemic**



**Annex 16: HemoCue Hemoglobin Quality Control Form**

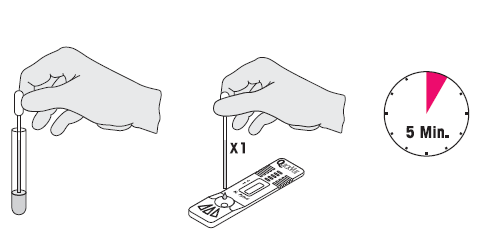


**Annex 17: Procedure for Checking H. pylori using the QuickVue Rapid Test Kit**

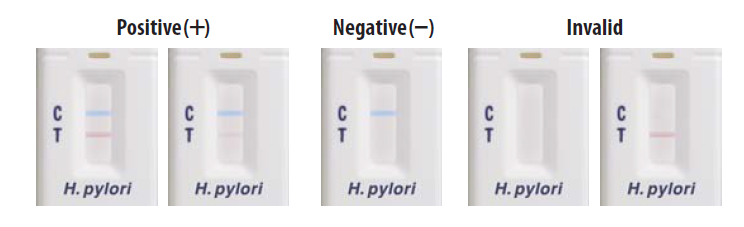
Helicobacter pylori is implicated in the etiology of a variety of gastrointestinal diseases, including gastric ulcer. The QuickVue H. pylori test detects H. pylori-specific IgG antibodies produced by individuals colonized or infected with the organism.

This test will only be used for Adolescent Boys and Girls 10-19 years.

Approximately 50µL of whole blood is added to the test cassette from the Purple Top Vacutainer. Set the timer for 5 minutes and then read the test.



**Results:**



**Annex 18: H. Pylori Status and Referral Slip**



**Annex 19: Referral Log for Participants Who Tested Positive for H. Pylori**



**Annex 20: Procedure for Urine Collection in the Field**

Give a labelled urine cup to each child 6-9 years and woman 15-49 years (both pregnant and non-pregnant) and explain the following before the urine collection:

1. Hands should be washed with soap and water.
2. The collection cup should not be opened until just before urinating.
3. The participant should leave the cap turned up (demonstrate this) while urinating, then immediately recap the filled container tightly.
4. It is most important that the inside of the container and the cap not be touched or come into contact with any parts of the body or clothing or external surfaces.
5. Collect the capped specimen from the subject and make sure that the cap is secured tightly.
6. Place each collection cup into cold box containing frozen gel packs.
7. After sample collection, record information for each specimen collected on the “**Specimen Control Form A**” (**Annex 3a**).
8. All urine specimens will be given to the Laboratory Technician for further processing.

**Annex 21: Procedure for Stool Specimen Collection**

Give each participant a stool collection kit containing a stool collection container, a disposable wooden spatula or spoon, and one pre-labelled stool cup and explain the following for stool collection, transfer, and storage:

1. Collect a stool sample using the stool collection container.
2. Using the disposable wooden spatula or spoon, transfer stool (3-4 scoops) to the stool cup.
3. Tighten the lid to of the cup tightly.
4. Dispose of all remaining stool and stool collection materials.
5. Ask the participant to store the stool cup in a cool, dark location inside the home until it is picked up by the team.
6. After sample collection, record information for each specimen collected on the “**Specimen Control Form A**” (**Annex 3a**).
7. All stool specimens will be given to the Pathologist for further processing.

**Annex 22: Kato Katz Technique**

***KATO KATZ QUANTITATIVE METHOD***

Instructions

1. The first morning stool specimen is the best and so you could give the poly pots the previous day to collection. There is sometimes a danger of participants sharing their specimens.
2. It has to be a fresh specimen. Hookworm results should be read immediately after processing as they could bust and disappear when left for long.
3. Keep processed slides in the shade and not in direct sunlight as they can peel off
4. Use protective clothing like laboratory coat and gloves to avoid contaminating yourself

Materials

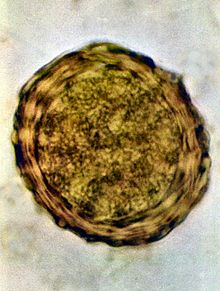
Metal template (50mg hole), fine Forceps, tally counter, stainless steel sieve, wooden spatula, glass slides, cellophane cover slips 22x30mm), gloves, tooth brushes, malachite green solution (100ml glycerol, 100ml water, 1ml 3% malachite green), liquid soap, basins, towels, tissue paper, polypots, labels, felt pen, compound microscope, “**Specimen Control Form C**” (**Annex 3c**).

Method

1. Sieve stool sample by pressing it through using wooden spatula
2. Fill the 50ml hole on template which is placed on a glass slide
3. Apply cellophane cover slip on the sample and place it upside down and press gently to spread the sample evenly on a bench covered with newspapers
4. For hookworms read slide immediately using x10 objective of a compound microscope. For other worms read after a number of hours or even next day
5. Record results after reading duplicate slides as follows: Total no of eggs in the 2 slides dived by 2 x 20 eggs per gram.

ASCARIS LUMRICOIDES ASCARIS LUMRICOIDES (INFERTILE)

(FERTILE)

HOOKWORM



TRICHURIS TRICIURA

1. [↑](#footnote-ref-1)