Note: This is an SOP for collection and processing of specimens from 2 types of health facilities, a diagnostic laboratory and a hospital/medical facility, performed by staff external to the health facility but working in collaboration with facility staff. The procedures and storage conditions described in this SOP may vary for other settings and facility-specific protocols.

Facility Survey study

Standard Operating Procedures

Collection and Processing of Specimens in the Facility Survey

Draft Version: May 2021

1 Purpose / Background:

1.1 Purpose of this SOP is to describe the general procedures for collecting facility-based residual specimens. Procedure will need to be tailored to each site. At each site there may be multiple facilities participating and providing residual specimens.

2 Scope / Applicability:

2.1 This SOP is applicable to all survey sites participating in the facility survey.

3 Roles / Responsibilities

3.1 Site Coordinator: Coordinates all staff at the site and communications with all facilities providing specimens. Primary point of contact for the central study investigators and central laboratory. One per site.

3.2 Specimen Collection Staff: Responsible for collecting specimens from each of the facilities, documenting details about each specimen in the register, transporting specimens to the site and reporting daily collections to central study team. Typically, one specimen collection staff per facility, however if collection from facilities occurs on alternative days the same specimen collection staff member could collect specimens from two different facilities.

3.3 Specimen Processing Staff: Responsible for processing, tracking, and storing specimens received from the facilities prior to shipment to central laboratory for testing. Typically, one specimen processing staff member per site.

3.4 Data Entry Staff: Responsible for entering specimen collection and processing data from paper registers to the central data collection system. May be a separate person or this role may be filled by one of the other staff members.

4 Prerequisites / Supplies Needed:

4.1 Study ID labels (all facilities):

4.1.1 Pre-printed standard labels for specimen collection tube and specimen collection register (2 labels per specimen)

4.1.2 Pre-printed cryolabels for cryovials/freezer tubes and specimen laboratory register (2 cryolabels per specimen)

4.1.2.1 NOTE: In settings where specimen tubes are labeled with personal identifiers and/or there are anticipated issues accessing linked data, an interim study ID may be used. These can also be pre-printed standard ID labels which are assigned to specimen collection tubes and specimen collection register.

4.2 Extra blank labels (as needed)

4.3 Black permanent marker for writing on blank labels *(one per facility)*

4.4 Tube rack *(one per facility)*

4.5 Gloves

4.6 Vaccine carrier *(one per facility)*

4.7 Log book (for recording notes during specimen collection process) *(one per facility)*

4.8 Specimen Collection Register *(one per facility)*

4.9 Specimen Processing Register *(one per site)*

4.10 Portable centrifuge *(one per facility at the site)*

4.11 Cryovials (0.5mL) *(one per specimen)*

4.12 Cryovials (2mL) *(one per specimen; only if run out of 0.5 mL vials)*

4.13 Pipettors (P100-P1000) and pipette tips

4.14 Cold/Freezer boxes, for storage *(one box per facility; number of boxes per site depends on specimen size [81 specimens fit in one box])*

4.15 Freezer Box Map

5 Specimen ID naming convention (See Appendix I for more details)

- **Study ID** numbers are assigned in the order specimens are obtained at that facility. At the time of processing each aliquot will be assigned a unique **Study ID** number. For each specimen there should be 4 pre printed labels for the specimen collection tube, Specimen Collection Register, cryovial/aliquot tube and Specimen Processing Register.
    - **NOTE**: In settings where specimen tubes are labeled with personal identifiers and/or there are anticipated issues accessing linked data, specimen collection staff assign an **interim ID** number at the time of specimen collection to each potentially age-eligible specimen collected. For each specimen there should be 2 interim ID labels, specimen collection tube and Specimen Collection Register.

6 Procedural Steps

Procedural steps will need to be tailored to each facility, attempting to integrate the collection of specimens within that facility's procedures. In this SOP two main types of facilities are described, a **diagnostic laboratory and a hospital/medical facility**. In both settings specimens and de-identified data will be obtained from the laboratory, with no interactions between the survey staff and the patients.

The minimum data needed for each specimen collected in the facility serosurvey are:

- Study ID (assigned by study team)
- Age (in years or months if less than 1 year)
- Sex
- Date of collection
- Date of retrieval

Additional data would be collected, if available and relevant for the question of interest:

- Facility where specimen was collected *(if specimens provided by diagnostic laboratory)*
- Antenatal care patient (Y/N)
- Location within hospital (inpatient vs outpatient department)
- Geographic information (e.g., village/ward; for specimens from a diagnostics center, the location of the originating facility may serve this same purpose)

Processing data needed for each specimen

- Date of processing
- Sera volume (ul)
- Hemolysis (none/ mild/ moderate or severe)
- Freezer box number and freezer box position.

Key considerations to discuss when selecting facilities and developing tailored procedures:

1. Is the facility public or private?
2. Who are the patients using the facilities (urgent care, primary level vs. tertiary level, representative of community or biased population, etc.)?
3. What types of patients typically have blood drawn at these facilities (inpatient/outpatient, chronic vs acute conditions, emergency, trauma, etc.)? What types of tests are typically performed on the specimens (routine CBC, specialized tests)?
4. For large hospitals, are there multiple laboratories, and if yes, which departments are served by each laboratory?
5. For hospital/medical facilities, are all specimens tested onsite or are some specimens sent to an external lab for testing?
6. How long does the facility retain the specimens (i.e. when can study team collect specimens)? What are the storage conditions while the facility is holding the specimens?
7. How are the specimens linked to the data (by name, by hospital ID, etc.)?
8. What information is written on specimen tube? What information is written in a paper-based log and/or computer database?
9. Is the above information available in a single database or separate sources? Will the survey staff be able to access the data, and if yes, how (e.g., via printouts from the laboratory staff, accessing the computer database)?
10. Is there the potential for specimens to be received from individuals living outside the district (study catchment area)? If yes, how to identify those specimens?

General considerations collection of specimens:

- Specimens should be obtained and processed within 48 hours of collection. In some settings this may be a longer duration if specimens are stored at +2 to 8 C but should not be longer than 96 hours between collection and processing. Duration between collection and processing will vary depending on the facility's laboratory procedures (how long they require specimens be held for), storage conditions and stability of antigen of interest.
- Eligible specimens are those that are within the specified age range, of sufficient volume (see below notes), and not hemolyzed.
    - Residual specimens should be at least 80-100 ul, but this is difficult for the specimen collection staff to estimate at the time of collecting the residual specimens.
    - If the number of residual specimens in the desired age group is limited at a facility, collection staff may collect all specimens with residual volume, acknowledging that some may be of too small volume after processing.
    - If the number of residual specimens in the desired age group is large at a facility and collection staff are only collecting the first X specimens (where X depends on specimen size and number of days specimens are collected per week), staff should consider volume of the residual specimen when pulling specimens for the survey. However, if there are certain tests performed at the facility that tend of be of higher volume, only selecting those specimens will systematically exclude certain patients. The team should discuss this with the facility and take into consideration when pulling specimens.
- Specimens must be de-identified before being transported to the site laboratory. Staff should affix the study ID label to the specimen tube in a way that covers any names, DOB, or facility ID numbers on the tube. You may also use a marker to blacken out any information not covered by the Study ID label.
- If log books are maintained with personal identifiers, pages should be removed and shredded monthly once all necessary data have been obtained. No facility identifiers should appear in the Specimen Collection Register.

Facility type 1: Diagnostic Laboratories

6.1 Specimen Collection Staff arrives at the facility and requests the laboratory staff provide the residual specimens collected in the past 96 hours that are ready to be discarded.

6.1.1 NOTE: As noted in general considerations, the duration between collection and processing will vary based on facility laboratory procedures, storage conditions and stability of antigen of interest.

6.1.2 There may be a dedicated location in the +2 to 8 C refrigerator or laboratory where the lab staff can store the specimens ready to be discarded for the collection staff to pull upon arrival.

6.2 If the patient's age is listed on the tube label collection staff should review all labels to identify eligible specimens:

6.2.1 Collection staff should check all residual specimens for eligibility (e.g., age eligible, serum not plasma not hemolyzed). All eligible specimens are transferred to the study tube rack.

6.2.2 Note: If site is capping the numbers of specimens obtained per day, only pull the number of specimens up to the cap. If there is the potential that future information may disqualify a specimen (e.g., from outside the district, based on the collection center data obtained in a later step), pull additional specimens. The number of additional specimens to be pulled may vary depending on how frequently specimens come from outside the district.

6.3 In a separate log book record the facility identification number, age, sex, and date of collection as study eligible specimens are being moved to the study tube rack. Specimens should be batched by date of collection. After recording the information, place the tube rack of age-eligible specimens into the +2 to 8 C refrigerator.

6.3.1 Note: This information only needs to be recorded in the log book if there is a need to link the specimens to a database at the facility to obtain additional information about the specimen (e.g., collection center). If not necessary, proceed to section 6.6 for labelling and documentation in the Specimen Collection Register.

6.3.1.1 If determined that the collection center is <u>outside the district or specimen is otherwise ineligible</u>, cross out the study ID in the log book and remove the specimen from the tube rack before proceeding to step 6.6.

6.4 If the patient's age is not listed on the tube:

6.4.1 Collection staff will need to cross-check the facility specimen identification number on the tube against either a printout of patient details or the laboratory computer database to confirm the patient is age-eligible before transferring the specimen to the study tube rack.

6.4.2 Alternatively, collection staff can review the printout or database prior to obtaining the specimens to identify which patients are age-eligible, noting down the facility specimen ID in a log book. Collection staff can bring the list of age-eligible patients, along with the Collection Register, pre-printed labels, tube rack, vaccine carrier, and gloves, to where the specimens are located to pull specimens for those individuals. Either method (6.4.1 or 6.4.2) is appropriate.

6.4.2.1 Note: If site is capping the numbers of specimens obtained per day recommend only noting down the facility specimen ID for the first patients in the database up to the number of capped specimens, plus 5 to 10 extras in case some specimens cannot be located (may need to adjust number of additional specimens depending on experiences at site and likelihood specimens will residual volume).

6.4.2.2 If reviewing the laboratory computer database, collection staff should also record details about the specimen in the log book, including date of collection, age, sex and facility name. This data can then be easily transcribed into the Specimen Collection Register for the final set of specimens.

6.4.3 Once all age-eligible specimens have been identified, proceed to section 6.6 to label the specimens and record details in the specimen collection register.

Facility type 2: Hospital/Medical Facility

6.5 Processes for a hospital/medical facility will vary depending on the facility. If working with a laboratory located within the facility, collection staff may obtain the residual specimens and identify those that are age-eligible following the same procedures as detailed for the diagnostic laboratory (starting from section 6.1), with minor modifications related to the types of data to be collected (e.g., if antenatal care patient, and outpatient department vs. inpatient department). If interfacing with the facility at the point of collection additional procedures may be needed; these will vary based on the facility.

6.5.1 NOTE: In settings where specimen tubes are labeled with personal identifiers and/or there are anticipated issues accessing linked data use an interim ID. Refer to Appendix II for more details.

Labeling and completing collection register

6.6 Take the first specimen from the tube rack of eligible specimen and assign an Study ID (see 'Specimen ID naming convention' section 5 and Appendix I):

6.6.1 Write the date of collection on the specimen label and collection register label (note: this should be the date of collection NOT date of retrieval)

6.6.2 Affix specimen label on tube. Make sure to cover patient name, facility ID, and interim ID (if applicable) with the Study ID label. You may also use a marker to blacken out any information not covered by the Study ID label.

6.6.3 Affix collection register label in corresponding column on register and complete the information in columns from the log (see 6.7).

6.6.4 Repeat for all study eligible residual specimens.

6.7 Specimen Collection Register: The following details should be recorded in the Specimen Collection Register (Appendix III). Note: some details may require a separate source or database at the facility; if not available at the time of completing the register leave the column blank and complete when data becomes available.

- Study ID
- Originating facility name/code (if specimens provided by a diagnostic laboratory)
- Age
- Sex
- Date of specimen collection (original date of collection)
- Date of retrieval from facility
- Comments on collection
- Additional variables where available/applicable:
    - Location within hospital (inpatient vs outpatient department)
    - Antenatal care patient
    - Geographic information (e.g., village/ward; for specimens from a diagnostics center, the location of the originating facility may serve this same purpose)

- Facility ID (varies across facilities: inpatient or outpatient number, lab ID, registration number etc.) *(remove and destroy this column from the register monthly; need to orient the columns on each page to allow for this column to be torn off)*

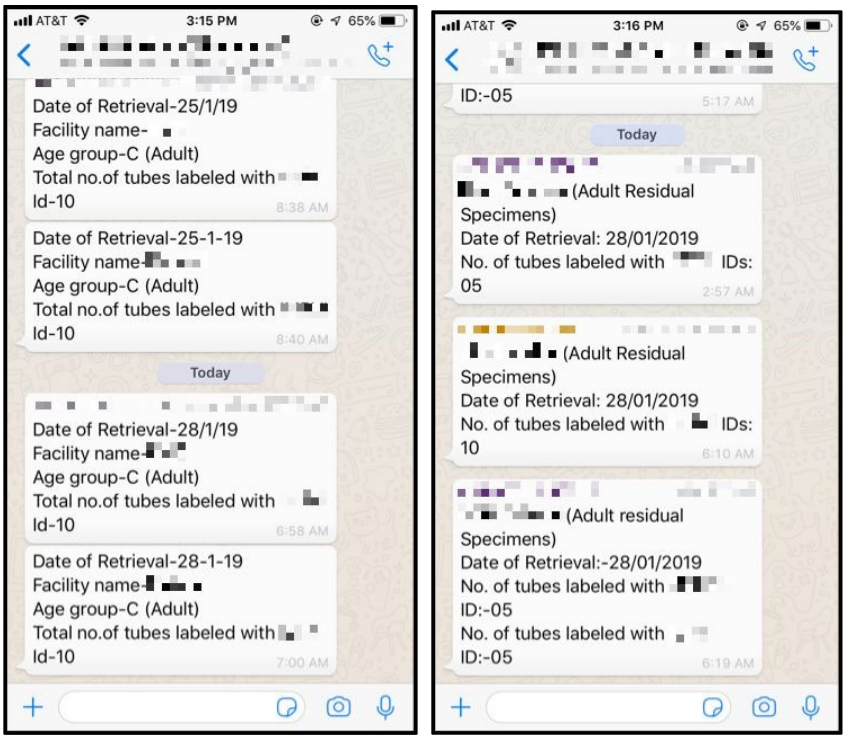
End of specimen collection process

6.8 Specimens should be centrifuged (10 minutes at 3000 RPM) at the collection facility using the portable centrifuge before transport back to the site. Check centrifuge specifications and ensure it is balanced.

6.9 Place all labeled and centrifuged specimen in vaccine carrier containing ice packs; prevent tubes from coming into contact with ice packs by placing inside a beaker with cotton.

6.10 Collection staff member sends a message to the WhatsApp group with the following information (see two examples below)

- Date of retrieval
- Facility name
- Age Group
- Total number of tubes labeled with interim IDs *(for those facilities assigning interim IDs)*
- Total number of tubes labeled with Study IDs
- Note: It may be of interest to track additional data about the facility during the first few weeks of the survey to support future planning, such as the number of adult residual specimens available each day.



6.11 Collection staff member transports specimens and the specimen collection register from the facility to the site laboratory for processing.

Specimen processing at the site

6.12 Upon receipt of vaccine carrier from collection staff review number of specimens against number of specimens reported by staff member. Assess cold chain of the specimens: Are ice packs frozen? Were tubes touching ice packs? If any problems, contact study coordinator immediately and record problems in lab register.

6.13 Preprinted labels (2 total): Write original date of specimen collection (copy from specimen collection label) on each label

- Lab register label
- Aliquot label (up to 500 uL)

6.14 Affix lab register label in the Lab Processing Register and aliquot label on a 0.5mL cryovial.

6.15 Assess hemolysis for each specimen and record in lab register (None, Mild, Moderate/Severe; refer to Appendix V for when grading hemolysis).

6.16 Put on gloves. Transfer 500 uL of sera into labeled 0.5 ml cryovial Any remaining volume should be discarded. Record estimated volume in lab register. Note: 2 mL tubes may be used in place of 0.5 ml cryovial if those tubes and corresponding freezer boxes are available, however maximum volume of sera specimen is 500 uL.

6.17 Place aliquot in freezer box. Record box number and position of cryovial in freezer box in the lab processing register and the freezer box map.

6.17.1 Separate freezer boxes should be used for each collection facility (see 6.20). Aliquots should be placed in the box in Study ID number order. If there are Study ID numbers without aliquots (i.e., specimen tubes were labelled at the facility but no remaining sera at the time of processing), leave blank spaces in the freezer box.

6.18 Store freezer boxes at -20^0C or lower.

6.19 Ensure Study lab processing register is completed with the following information for each specimen (Appendix IV):

- Study ID (affix label)
- Processing date
- Sera volume (ul)
- Hemolysis (None, Mild, Moderate/Severe)
- Aliquot freezer box number
- Aliquot freezer box position
- Comments on processing

6.20 Label freezer box

- All specimens will be stored in 1 aliquot
- Freezer box ID scheme (right), where the first digit is the site [4=Site 1, 5=Site 2, etc.], the second digit is the facility, and the boxes within a facility proceed alphabetically [A, B, C,…]

Facility Name	Freezer Box Number
Site 1	
Facility 1	41A, 41B, 41C...
Facility 2	42A, 42B, 42C...
Facility 3	43A, 43B, 43C...
Facility 4	44A, 44B, 44C...
Site 2	
Facility 1	51A, 51B, 51C...
Facility 2	52A, 52B, 52C...

Data entry

6.21 Data will be entered into a KoBoToolBox data system. Two separate forms will be used:

- Specimen Collection: data entered from Specimen Collection Register
- Specimen Processing: data entered from Lab Processing Register

Appendix I. Specimen ID naming convention

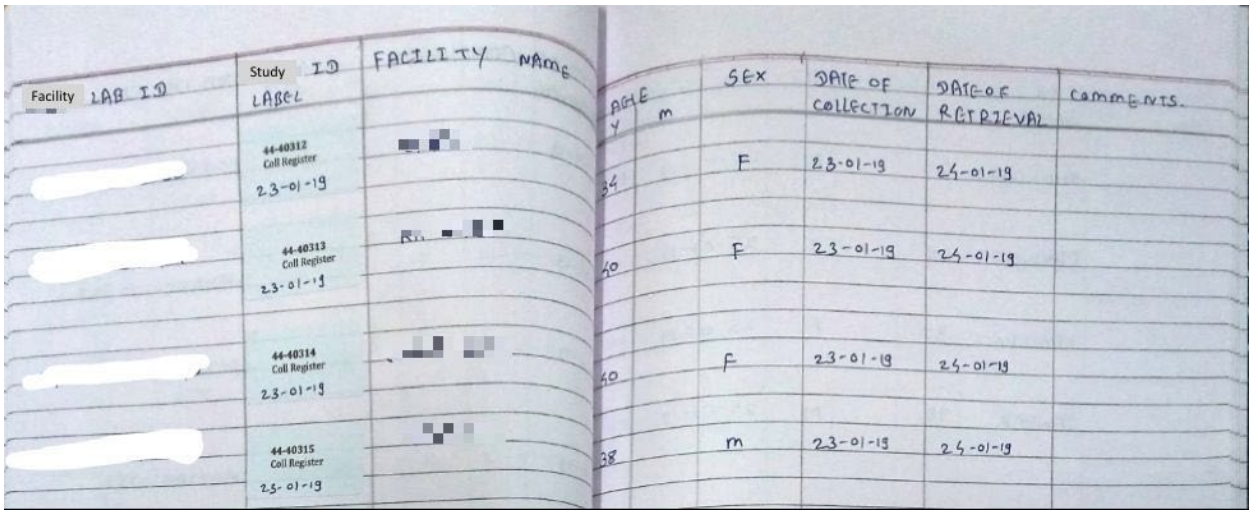
- **Study ID (applicable in ALL facilities)** numbers are assigned in the order specimens are obtained at that facility: SF-FXXXX
    - S: Site identifier
    - F: Facility number, ordered within each site
    - FXXXX: Individual ID (0001-9999), where the first digit reflects the facility identifier.
  - For each specimen there should be 4 pre-printed labels
    - Specimen collection tube *(used at facility lab)*
    - Specimen collection Register *(used at facility lab)*
    - Cryovial/Aliquot tube *(used at site lab)*
    - Specimen processing Register *(used at site lab)*
- **Interim ID number:** For medical facilities where specimens are not assigned a unique ID (labeled with patient name etc) specimen collection staff assign an **interim ID** number at the time of specimen collection to each potentially age-eligible specimen collected: FI- XXXXX (Refer to section 6.5 for more details)
    - FI: 2 letter facility initials
    - XXXXX: individual ID (00001-99999)
  - For each specimen, there should be 2 interim ID labels
    - Specimen collection tube *(used at specimen collection point)*
    - Collection Register *(used at specimen collection point)*

Appendix II. Detailed procedures when using an interim ID

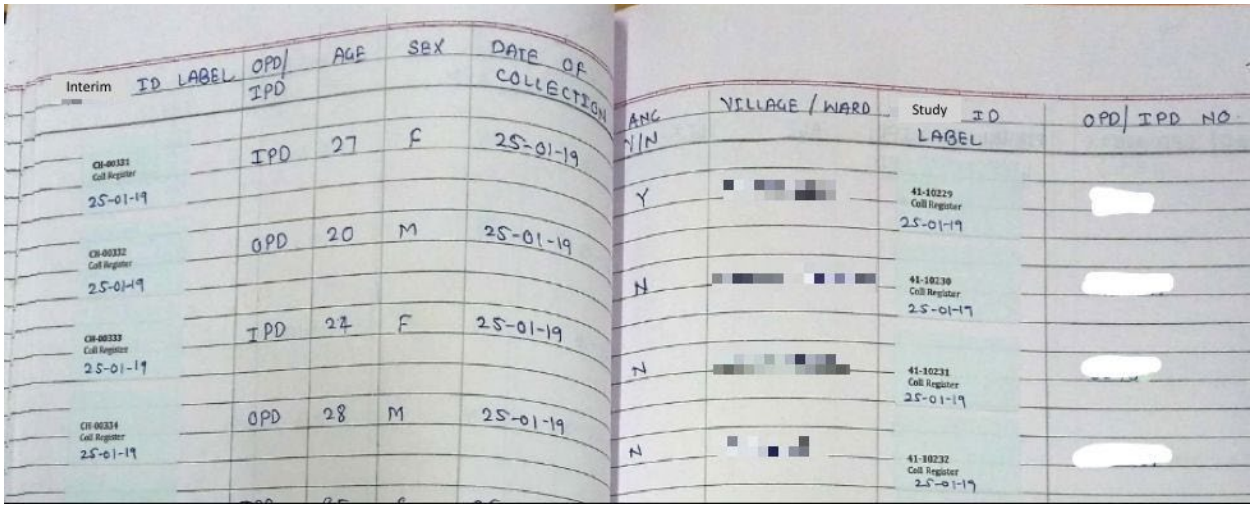
- A collection staff member will need to be positioned in the laboratory during the facility's collection hours to record details in the Specimen Collection Register and assign an interim survey ID for each specimen collected from an age-eligible patient to link the specimen to the data. The specimens are then tested by the facility laboratory and any specimens with residual volume are set aside by the laboratory staff for the collection staff to collect at the end of the day or the following morning.
- The interim survey ID is a single ID assigned to every age-eligible specimen collected, typically pre-printed on standard labels (not cryolabels). This ID is used to link any residual specimens available after testing to the data recorded in the Specimen Collection Register at the time of collection.
- Refer to section 6.7 for details on data to be recorded in the Specimen Collection Register. Details should be abstracted by the collection staff using the register completed by facility staff at the time of collection.
- When the residual specimens become available (at end of day or following morning):
    - Check the interim survey ID on the residual specimen tube against specimen collection register to confirm the patient is age-eligible.
    - If confirmed, assign a Study ID to the specimen. Refer to sections 5 and 6.6 for more details on labelling.
    - For those records in the Specimen Collection Register with an interim survey ID that were not subsequently assigned an Study ID, the survey staff should draw a line through the record indicating the specimen was not enrolled in the survey.

Appendix III. Specimen Collection Register

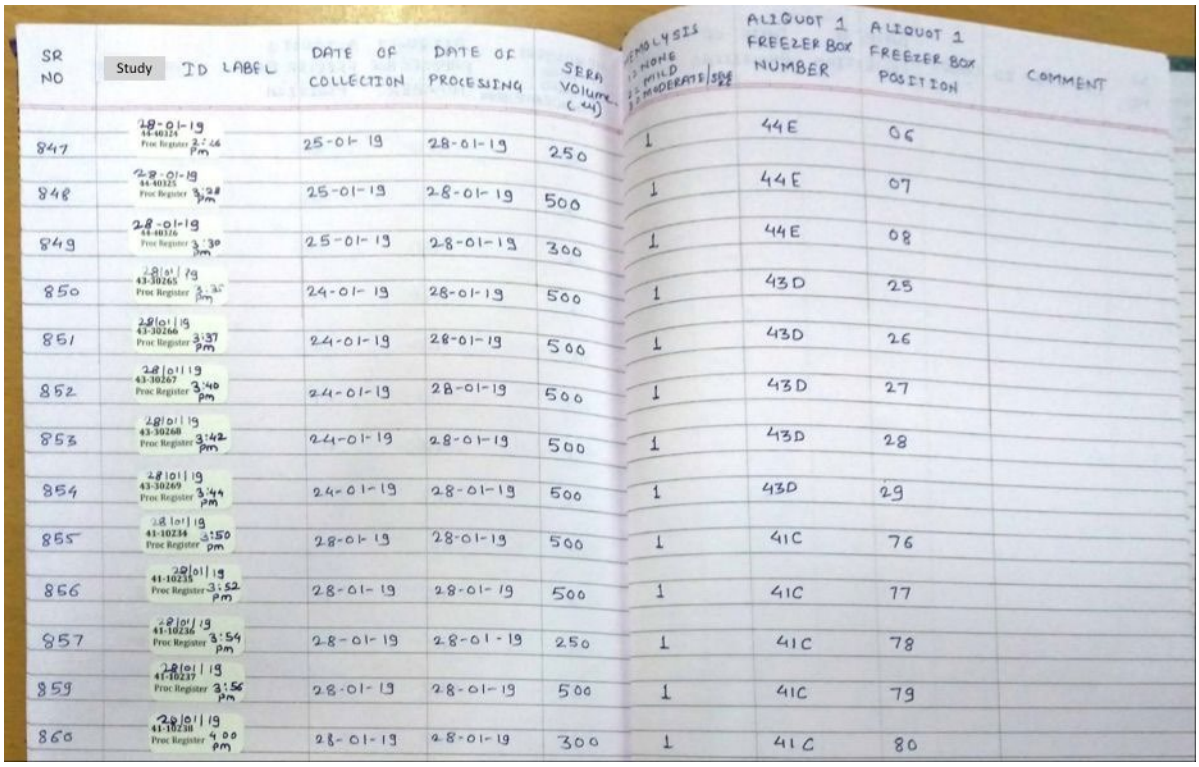
Diagnostic Laboratory



Health Facility



Appendix IV. Lab Processing Register



SR NO	Study ID LABEL	DATE OF COLLECTION	DATE OF PROCESSING	SERA VOLUME (µl)	HEMOLYSIS 1: NONE 2: MILD 3: MODERATE/SEVERE	ALIQUOT 1 FREEZER BOX NUMBER	ALIQUOT 1 FREEZER BOX POSITION	COMMENT
847	28-01-19 44-40124 Proc Register 2:46 pm	25-01-19	28-01-19	250	1	44E	06	
848	28-01-19 44-40125 Proc Register 3:28 pm	25-01-19	28-01-19	500	1	44E	07	
849	28-01-19 44-40126 Proc Register 3:30 pm	25-01-19	28-01-19	300	1	44E	08	
850	28/01/19 43-30265 Proc Register 3:35 pm	24-01-19	28-01-19	500	1	43D	25	
851	28/01/19 43-30266 Proc Register 3:37 pm	24-01-19	28-01-19	500	1	43D	26	
852	28/01/19 43-30267 Proc Register 3:40 pm	24-01-19	28-01-19	500	1	43D	27	
853	28/01/19 43-30268 Proc Register 3:42 pm	24-01-19	28-01-19	500	1	43D	28	
854	28/01/19 43-30269 Proc Register 3:44 pm	24-01-19	28-01-19	500	1	43D	29	
855	28/01/19 41-10234 Proc Register 3:50 pm	28-01-19	28-01-19	500	1	41C	76	
856	28/01/19 41-10235 Proc Register 3:52 pm	28-01-19	28-01-19	500	1	41C	77	
857	28/01/19 41-10236 Proc Register 3:54 pm	28-01-19	28-01-19	250	1	41C	78	
859	28/01/19 41-10237 Proc Register 3:56 pm	28-01-19	28-01-19	500	1	41C	79	
860	28/01/19 41-10238 Proc Register 4:00 pm	28-01-19	28-01-19	300	1	41C	80	

Appendix V. Hemolysis grading