Manual for the

Field level laboratory component

Of Integrated Biological and Behavioural Assessment (IBBA)

In Tamil Nadu, Andhra Pradesh, Maharashtra, Manipur and Nagaland, India

Note: The manual of the behavioural team has used "respondents" in place of "participants" in this manual

FIELD LEVEL SOPs FOR THE LABORATORY COMPONENT

CONTENTS

ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
BMGF	Bill and Melinda Gates Foundation
CBO	Community Based Organization
CRS	Chain Referral Sampling
CT	*Chlamydia trachomatis*
DBS	Dried Blood Spot
EC	Endocervical
ELISA	Enzyme Linked Immunosorbent Assay
FHI	Family Health International
FSW	Female Sex Worker
FVU	First Voided Urine
GC	*Neisseria gonorrhoeae*
FSW-BB	Female Sex Worker – Brothel Based
FSW-NBB	Female Sex Worker – Non Brothel Based
FSW-HB	Female Sex Worker – Highway Based
HD	*Haemophilus ducreyi*
HBV	Hepatitis B Virus
HBC	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IBBA	Integrated Behavioral and Biological Assessment
IDU	Injecting Drug User
LT	Laboratory Technician
M/E	Microscopic Examination
MSM	Men who have sex with men
MSW	Male Sex Worker
NACO	National AIDS Control Organization
NARI	National AIDS Research Institute
NG	*Neisseria Gonnorhoeae*
PA	Particle Agglutination
PCR	Polymerase Chain Reaction
RPR	Rapid Plasma Reagin
STI	Sexually transmitted infection
TP	Treponema pallidum
TPHA	Treponema *pallidum* Hemagglutination Assay
USTT	Urine Specimen Transportation Tube
VCT	Voluntary Counselling and Testing

CHAPTER 1

INTRODUCTION

The purpose of the Integrated Bio-Behavioural Assessment (IBBA), a survey, is to gather data for impact monitoring and evaluation of the Avahan India AIDS Initiative funded by the Bill & Melinda Gates Foundation in 71 districts of 6 States and five highway sites. This document gives standard operating procedures for the laboratory component of the IBBA Round 2 survey. Details of the survey and its behavioural component are given elsewhere.

The survey is being carried out in 29 districts of 5 states and the national highways.

Study Population:

In the five states the study population comprises of:

- Female Sex Workers - Brothel Based (FSW-BB)
- Female Sex Workers - Non-Brothel Based (FSW-NBB)
- Male Sex workers (MSW)/Men who have sex with men (MSM)
- Male clients of Female Sex Workers
- Injecting Drug Users (IDU)

In the national highway segments the study population comprises of:

- Truckers and Assistants
- Female sex workers Highway-based (FSW-HB)

Site of the study:

The site where the actual study would be carried out to interview the participants and take biological specimens has been chosen after extensive and careful preparation in consultation with the HIV/AIDS programme staff from NACO and State AIDS Control Societies, the district authorities, the various study partners and the community from where the participants have been selected.

Staff:

The team members for carrying out the study at the field level will be:

- Medical Officer – leader and overall supervisor of the biological (laboratory and clinical) team
- Supervisor
- Community liaison worker
- Interviewers (3 in a team)
- Laboratory technician

Depending on the area there might be more than one team working in one district. The job responsibilities of each member have been defined but all members work as a team to carry out the study while maintaining appropriate rapport with the community, ensuring co-operation from the study participants and maintaining cordiality at the site.

Job responsibilities of all members are given elsewhere and only of the laboratory technician (LT) are listed here:

Job responsibilities of the laboratory technician

- Ensuring adequate stock and maintenance of all laboratory consumables and non-consumables required for the biological component of the survey
- Correct labelling of all specimens
- Collection of biological specimens (blood, urine and dry blood spot (DBS)) after appropriate instructions to the participants
- Correct documentation of all specimens (lab forms, etc.)
- Management of gel packs and transportation of boxes
- Proper packaging of all specimens
- Assist the medical officer in collection of specimen from the ulcer
- Ensuring proper waste disposal
- Co-ordinating and assisting others in the field team

Chapter 2

Biological specimens to be collected from the study participants

This chapter gives the overall view of the tests to be performed and movement of the specimens and dealing with the participants.

Biological tests:

The tests to be conducted on all these samples are as per the table below:

S. No.	Name of the disease/symptom	Test to be done	Specimen to be collected	Percentage of samples to be tested	Level where the test will be done
1	Syphilis	Rapid Plasma Reagin (RPR) (titration)	Blood	All samples	District
2	Syphilis	TPHA	Blood	All RPR Reactive samples	State
3	Syphilis	ELISA for antibody to TP (Treponistica)	Dried blood spots (DBS)	All DBS samples	State
4	HIV (Human Immuno-deficiency virus) (prevalence)	ELISA Screening Confirmatory	Blood/DBS	First ELISA on all, second ELISA on all samples positive by the first ELISA	State (Discordant test to be confirmed at NARI)
5	HIV (incidence)	BED-CEIA	Blood/DBS	All samples positive by both the prevalence HIV ELISAs	NARI
6	HSV2 (Herpes Simplex Virus	ELISA	Blood/DBS	On subset – 10% of all samples	State
7	NG (Neisseria Gonorrhoeae)/CT (Chlamydia Trachomatis)	Transcription Mediated Amplification (TMA)	Urine	All samples	NARI and other site
8	HBV	For HBsAg	DBS	All DBS samples	RMRC
9	HCV (Hepatitis C Virus)	Screening test & RIBA (confirmatory	DBS	All DBS samples	RMRC

		test)			
10	GUD (Genital ulcer diseases) (only on those reporting an ulcer)	mPCR for TP, HD and HSV	Genital swab	All swab samples collected from external genital ulcers if reported	NARI

In IDU respondents, the Dried Blood Spots (DBS) instead of blood will be used as the specimen to carry out the tests for HIV, Syphilis, HBV, HCV, and HSV2. DBS will not be collected from the other participants.

Specimen collection:

The study respondents from whom the sample is to be collected would already have been interviewed by the interviewer and undergone clinical examination (if required) before reporting for sample collection. He/she would have given her/his consent for the laboratory testing. It is important to remember that the participant in the study has the right to refuse to give the sample. It is important that the procedure is explained to her/him once before the actual collection of the specimen. If s/he wants to know more about the test results, s/he should be guided to the supervisor.

The specimens to be collected from the participants are as follows:

1. Blood sample from all study participants (except IDUs)
2. Urine sample from all study participants
3. DBS from only IDUs
4. Ulcer swab only from those who report an external genital ulcer.

All specimens except for the swab from the genital ulcers would be collected by the LT. The swab will be collected by the medical officer.

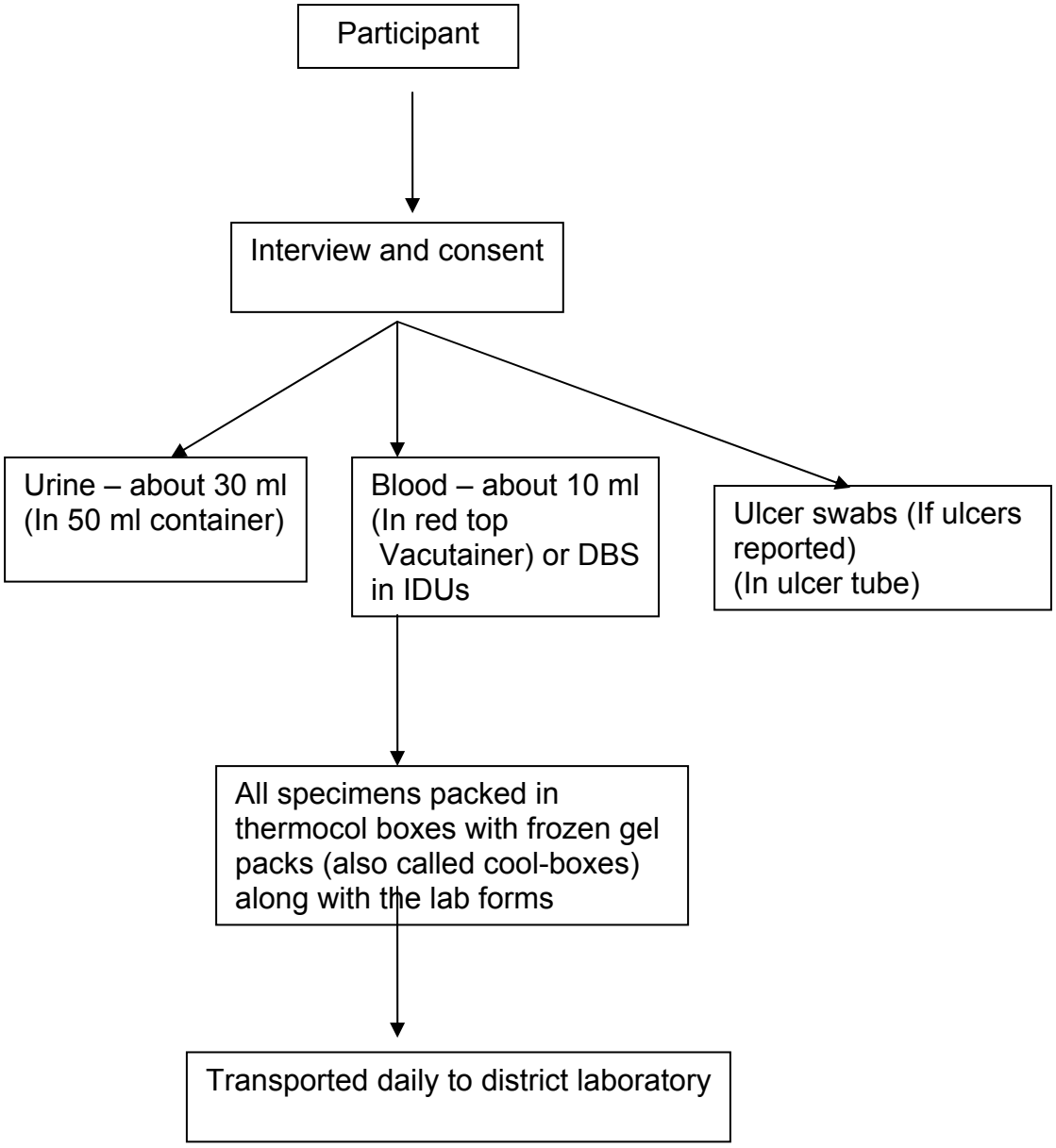
The specimens collected from the field will be transported to the district, on to the state and then on to the national level. During the transportation appropriate conditions would be maintained to preserve the specimens, to avoid contamination and avoid errors in recording and reporting. Also, each laboratory would follow certain standards of quality control and would be supervised by the level above.

Levels of laboratories

There are 3 levels of laboratories in the IBBA

1. National level – National AIDS Research Institute, Pune
2. State level –at each state ICMR Institute
3. District level

FLOW CHART FOR COLLECTION OF SPECIMENS



Chapter 3

Organization of work place for specimen collection in the field

At the beginning of each day the LT should ensure that adequate stock of consumables and non-consumables are available for the day's work. In a given day it is expected that each interviewer would be able to interview three-four participants. As three interviewers are expected in each team, it is expected that about 10-12 participants would come for collection of biological specimens. Since two (blood and urine), and sometimes three (ulcer swab) specimens have to be collected from each participant, the LT should ensure provision of the following number of consumables/instruments and equipments (provision has been made for breakage, loss etc.)

List of supplies for collection of samples from 15 participants or for approximately one day

1. Vacutainer tubes for blood –15
2. Needles – 15
3. Needle holder - 2
4. Tourniquets – 2
5. Alcohol swabs – 30
6. Containers for collection of urine (50 ml containers) – 15
7. Urine specimen transport tube with disposable pipette – 15
8. Dacron swabs - 4
9. Tubes for Dacron swabs – 4
10. Sterile cotton balls – 4
11. Sterile normal saline- 100ml
12. Thermocol boxes with sponges and thermometers - 3
13. Lab forms – 2 (plus extra)
14. Scissors – 1 pair
15. Packaging tape – 1 roll
16. Cello tape – 1 roll
17. Permanent markers – 1
18. Tissue roll for wiping the specimen containers dry and for cleaning the work place - 1
19. Gel packs (frozen) – 6 (2 for each box)
20. Disposable gloves – 35 pairs
21. Small zip-lock bags – 40 (one each for packing one specimen)
22. Large zip-lock bags – 3

23. Black bags for disposal – 3
24. Autoclave bags – 4
25. Labels (with participant's ID numbers) – 15 sets (one ID number will be pre-printed on 15 labels and would be used for the questionnaires, log forms, various specimens etc. A few will be extra to compensate for loss and damage).
26. Sharps disposal container

(Following items are for IDUs where DBS card required and)

27. DBS paper (No. 903, Schleicher and Schuell) – 30 (two DBS papers required per subject) with envelope
28. Lancet – 30 (two lancets required per subject)
29. Large low gas permeable ziplock bag –2
30. Dessicant pouches-15
31. Humidity Indicator card -2

Note: The field workers are instructed to maintain a minimum stock levels of 3 days to overcome unforeseen interruption of regular supplies from the district:

Besides this, the following should be ensured:

1. Chair for the participant
2. Chair for the LT
3. Chair for the medical officer
4. A working table
5. Cot for examination
6. Screen for privacy during examination
7. Lamp for adequate lighting
8. Newspaper or brown paper
9. Waste disposal bins (3)
10. Needle destroyer (manual)
11. Arrangement for running water
12. Soap for washing hands
13. Cabinet for keeping the forms and other stationery
14. Cabinet for keeping the stock of equipment/lab consumables
15. Small desk for keeping the thermocol box for transporting the specimens

16. Arrangement for post-exposure prophylaxis for the LT (Details are given in the medical officers' manual)

To avoid melting of the gel packs, shaded and cool lab site is best. The work place should be adequately lighted.

Consumables should be requisitioned from the District Laboratory by filling in the Field requisition form as below and handing it over to the person taking the samples to the district Laboratory.

Date----------
Field team ----------
Name of the supervisor ----------
Name of the doctor ----------
Name of the laboratory technician ----------
When was the last supply received ----------
-

Article	Total Quantity received till now	Total Quantity consumed till now	Quantity on hand at present	Quantity requested	Quantity actually received**

Instructions for filling the form:

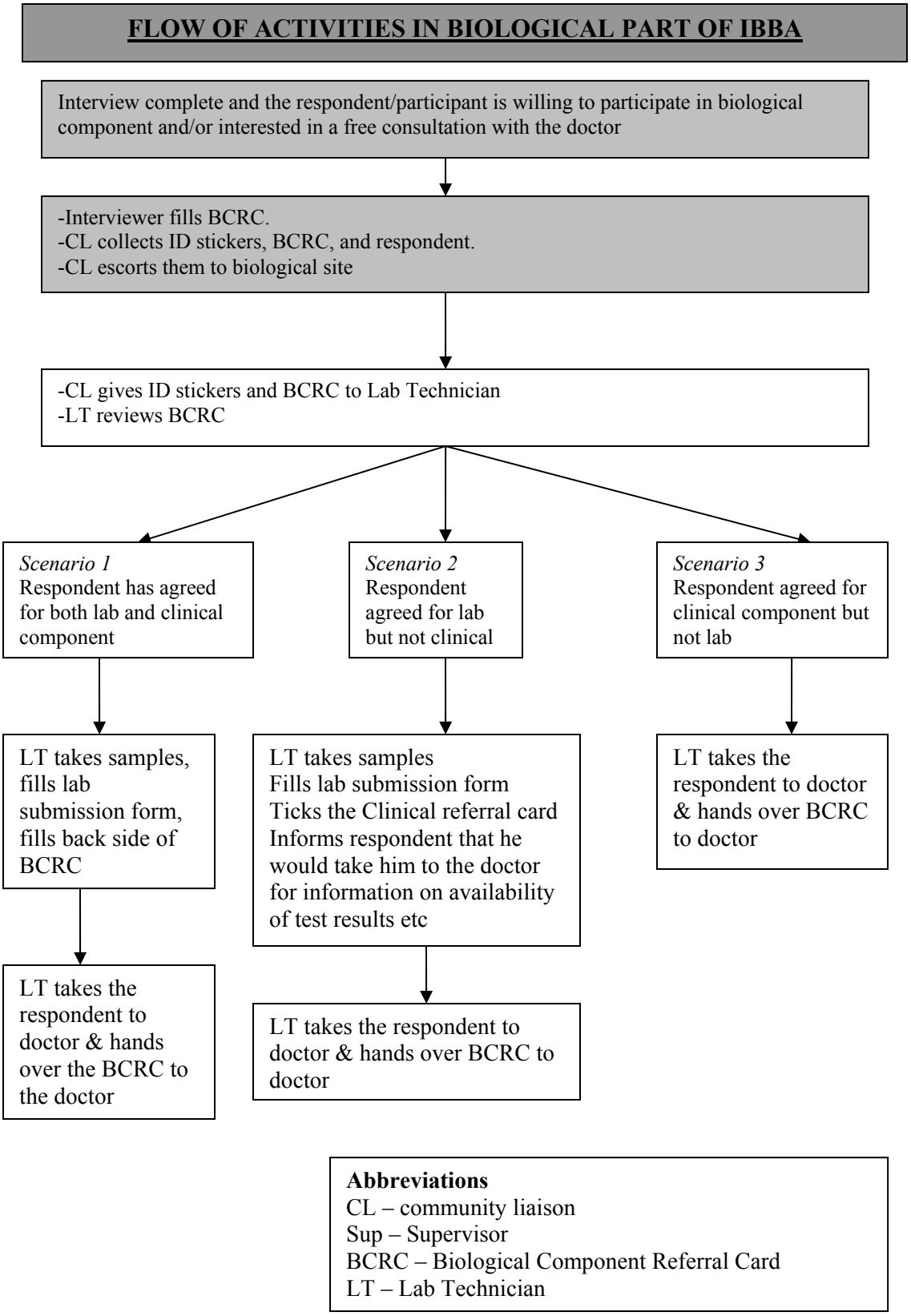
- Please write nil where required instead of leaving the cell blank
- At the time of requesting the drugs do not fill the last column
- Please fill in duplicate
- Send one copy to the district and retain one as office copy with you
- **After receiving the supplies enter the quantity received in the last column in the office copy with you.

Chapter 4

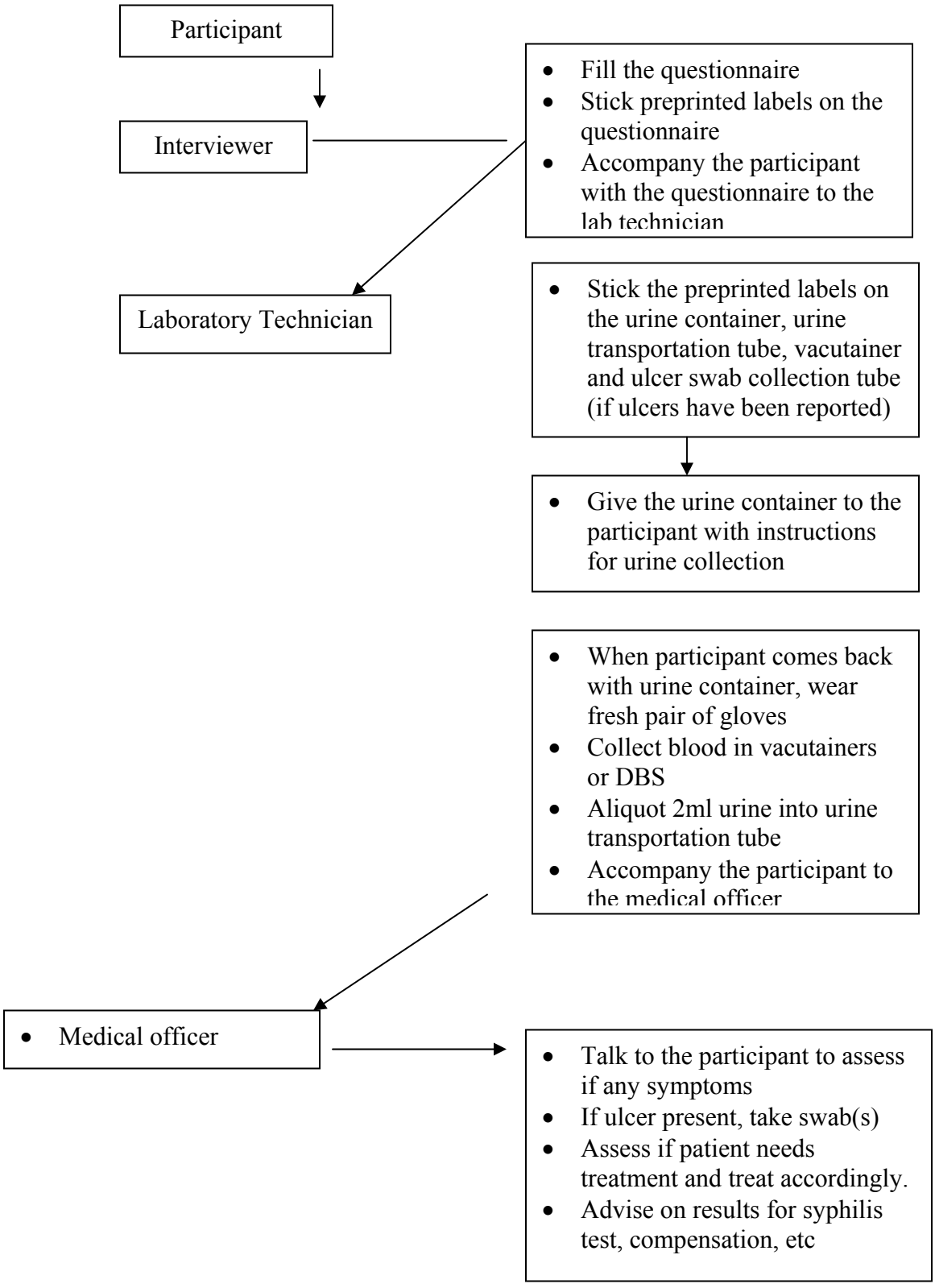
Procedure for collection of various specimens

UNIVERSAL PRECAUTIONS SHOULD BE ADHERED WHILE HANDLING ALL SPECIMENS including packaging/unpackaging/during testing etc. (see WHO guidelines at the end of the manual)

The participant would come to the laboratory technician accompanied by the interviewer. The participant would have first met the interviewer. (S/he would have given consent and talked to the interviewer, the details of which are given elsewhere). The interviewer would have a set of 15 pre-printed labels with a unique identification number of the participant. The interviewer would paste two of these labels on the questionnaire. After the interview, the interviewer would accompany the participant to the laboratory technician and introduce them. In addition, the interviewer would hand over the remaining set of pre-printed labels to the laboratory technician for use on the containers and tubes, etc.


FLOW OF ACTIVITIES IN BIOLOGICAL PART OF IBBA
Interview complete and the respondent/participant is willing to participate in biological component and/or interested in a free consultation with the doctor
-Interviewer fills BCRC.
-CL collects ID stickers, BCRC, and respondent.
-CL escorts them to biological site
-CL gives ID stickers and BCRC to Lab Technician
-LT reviews BCRC
Scenario 1
Respondent has agreed for both lab and clinical component
Scenario 2
Respondent agreed for lab but not clinical
Scenario 3
Respondent agreed for clinical component but not lab
LT takes samples, fills lab submission form, fills back side of BCRC
LT takes samples
Fills lab submission form
Ticks the Clinical referral card
Informs respondent that he would take him to the doctor for information on availability of test results etc
LT takes the respondent to doctor & hands over BCRC to doctor
LT takes the respondent to doctor & hands over the BCRC to the doctor
LT takes the respondent to doctor & hands over BCRC to doctor
Abbreviations
CL – community liaison
Sup – Supervisor
BCRC – Biological Component Referral Card
LT – Lab Technician

WORK FLOW FOR COLLECTING SAMPLES



During the collection of specimens it is of utmost importance to ensure the following:

1. Maximum efforts should be made to ensure that study participants are comfortable and do not feel anxious during the entire period they are at the site for interview and specimen collection. In general, it is good to smile and make eye contact with the participant throughout the specimen collection.
2. Before starting the blood collection procedure, ask the participant if they would feel more comfortable if the community liaison person were present during the blood collection.
3. Participants should be made to understand the procedure that is going to be undertaken. Use common language to describe what will happen (e.g. describe the amount of blood required as a little more than a teaspoon) and ask the participant if they have any questions. While the blood collection is taking place, continue to talk and explain what you are doing to reassure the participant.
4. All team members should understand that participants have the right to refuse any part of the study and they have the right of leaving the study at any time. If a participant seems very anxious during the process, ask them again if they have any doubts and let them know it is alright if they want to leave the study at that time.
5. All questions from the participants should be answered truthfully and respectfully.
6. At the time of leaving the study the participants should have received appropriate medications (and referral if required). The LT should make sure the participant is met by the medical officer on the team.
7. At the time of leaving the study the participants should have a clear understanding that they can collect the test results of syphilis and they should know how and where to collect it from. All members of the team, including the LT should be familiar and be able to explain the process of how participants can get their syphilis test results. The LT should reinforce the importance of the participant returning for their syphilis test result.
8. If the participant reports an external ulcer which cannot be seen during medical examination at the field site, s/he should be referred to the Avahan clinic.
9. If the participant desires, s/he should be suitably guided to the nearest VCTC or the Avahan clinic and be offered an escort to assist if s/he wants one.
10. After the specimen collection, especially after giving blood, the participant should be observed for any feeling of giddiness or dizziness. Ask her/him if s/he is feeling alright. If not ask her to remain seated and ask the doctor to check if required. Participants should be offered refreshments before leaving.

Universal precautions should be practiced at all times while ensuring that participants do not feel awkward and that they understand the procedure.

Other instructions before starting specimen collection

- LT should wear gloves all the times while collecting and handling the samples. Use one pair of gloves for each participant and change gloves in between participants. Before wearing the gloves, the participant should be informed that wearing gloves for sample collection will be protective for both the participant as well as the LT.
- The order of collecting blood, urine and swab specimens is as given in the flow diagram
- Before starting the collection of various specimens from the participant, cover the desk with some newspaper or brown paper. This is to prevent the soiling of the desk. The newspaper or the brown paper should be discarded at the end of the day or if it gets soiled.
- Before starting the collection of various specimens from the participant, stick the labels with the participant's ID on the following tubes
    - One vacutainer tube
    - Urine collection container
    - Urine specimen transportation tube
    - Swab tube (if required)

List of labels required for one participant (at all levels)

	Labels required	
S. No.	**At field level**	
1	Questionnaire	
2	Consent	
3	Biological component referral card (BCRC)	
4	STI referral	
5	Clinical Format	
6	Urine container	
7	Urine transportation tube	
8	Vacutainer	
	At district level	
9	Serum: RPR and TPHA	
10	Serum: HIV and HSV 2	
11	Serum: QC	
	At state level	
12	Urine QC	
13	Blank	
14	Blank	
15	Blank	

A) Collection of First voided urine (FVU) specimen

Participants**:** All study participants

Materials required:

1. One 50 ml urine collection container.
2. One Urine specimen transportation tube (USTT) with disposable pipette.
3. One small zip lock bag for packing the specimen transportation tube.
4. Pre printed labels.(set of 15 labels for each study no)
5. Disposable gloves.
6. Scissors

Procedure for collection of urine specimen:

1. Same procedure is followed for both male and female participants.
2. Ask the female participant if she is having menstrual periods. If yes, do not take urine specimen from her. (Mention this in the remarks column of lab submission form – please see later.)
3. Ask the participant when s/he last passed urine. (At the time of specimen collection at least 2 hours should have elapsed since s/he last passed urine.) If s/he last passed urine less than two hours ago then the participant should be requested to wait.
4. Explain to the participant how to collect the FVU, emphasizing the need to include the first few drops and to wipe the outside of the container with tissue paper.
5. Stick the label with the participant's unique number on the container
6. Show the line at the 30 ml level to the participant if it is already drawn on the container, if not please draw a line and show it to her/him. Ask her/him to collect urine till about that level, and return the container.
7. When the container is returned asked the participant to place it on a piece of tissue paper on the table and check to see that the volume is about 30 ml.
8. With gloved hands transfer 2 ml of urine with the help of disposable pipette (provided with USTT) to Urine specimen transport tube (USTT). The correct volume of urine has been added when the fluid level is between the black fill lines on urine transport tube label. (Before doing this step, take her blood specimen and wearing the same gloves, do the urine transfer). Close the lid of the tube.
9. Stick the label with the pre-printed ID no. of the participant, on the urine transport tube. DO NOT COVER WINDOW INDICATING TUBE VOLUME.
10. Throw away the urine container after screwing on the lid with remaining urine in the autoclave bags.

11. Place the urine specimen transport tube in the small zip-lock bag and zip the bag tightly.
12. Place this small zip-lock bag in cold thermocol box with gel packs (cool box).

B) Collection of Blood specimen

Participants**:** All Male and Female except the IDUs

Materials required:

1. Skin disinfection: Alcohol swabs (pre-packed)
2. Disposable gloves
3. Tourniquet
4. One vacutainer with red-top
5. One needle and a holder
6. Pre printed labels(set of 15 labels for each study no)
7. Cello-tape
8. One small zip lock bag
9. Scissors
10. Manual needle destroyer
11. Sharps Disposal Container

Procedure

Caution: If the respondent has any skin lesions wear double gloves and avoid touching the lesions as far as possible.

1. Use a sterile vacutainer tube with red top.
2. Label the side of the vacutainer tube with the participant's ID number. (Label should be stuck on the side of the tube and not on the cap.)
3. About 10 ml blood will need to be collected
4. Open the needle package but do not remove the needle shield. Attach the needle onto the holder.
5. Next, select the site for venipuncture.
6. Apply tourniquet a hand's breadth above the elbow. Instruct the patient to clench the hand to make a fist and unclench – to repeat this thrice so that the vein stands out.
7. Wear gloves.

8. Inspect the bend of the elbow to locate a suitable vein. Palpate the vein with the finger tip(s) to determine the direction of the vein, to estimate its size and depth, and any tendency to roll.
9. Cleanse the skin of the puncture site using an alcohol swab. Allow alcohol to dry. Do not palpate venipuncture site after cleansing.
10. Remove the needle shield. Perform venipuncture with the arm in a downward position.
11. Insert tube into holder. Push the stopper of the tube onto the needle. Tube will retract slightly. LEAVE IN THIS POSITION.
12. Remove tourniquet as soon as blood appears in the tube.
13. During the procedure do not allow the contents of the tube to come in contact with the stopper or the end of the needle.
14. Ten ml of blood has to be collected. If the blood stops flowing before 10 ml has been collected, second venipuncture will have to be done.
15. Place an alcohol swab over the puncture site and withdraw the needle from the vein. Do not press down on the needle. Instruct the participant to press the cotton ball over the wound for three to five minutes with the forearm folded upwards.
16. After the venipuncture, the top of the stopper may contain residual blood at the puncture site. Proper precautions should be taken when handling tubes to avoid contact with blood droplets.
17. Dispose off the holder if it becomes contaminated with blood.
18. Place the vacutainer with blood sample in the small zip lock bag and seal the bag. Leave it for about 20 minutes to allow the blood to clot.
19. Place this zip-lock bag with vacutainer in cold thermocol box with gel packs (cool box).
20. Check patient to be sure that bleeding has stopped; apply band-aid if necessary.
21. DO NOT RESHIELD the needle. Insert needle up to the plastic hub into the manual needle destroyer, which will cut the needle away from the plastic hub. Dispose of the plastic hub into the medical waste sharps container.
22. If the tube containing blood sample breaks, avoid contact with exposed skin and follow proper procedures for the cleanup and disposal of infectious waste.

C) Collection of ulcer swab

To be collected by: Medical officer

Materials required:

1. Dacron swabs – one per participant per ulcer
2. Tube for collection of swabs
3. Disposal gloves
4. Labels with the participant's ID no.
5. Cello-tape
6. Small zip-lock bags
7. Sterile cotton wool balls and sterile saline solution
8. Scissors

Procedure:

If the participant reports an ulcer, medical examination will be done by the medical officer and an ulcer swab will be collected.

1. Stick the label with the participants study number on the sterile screw-capped swab container in which the swab will be transported.
2. Clean the ulcer with cotton wool dipped in sterile saline (remove any crust or dead skin).
3. Gently hold the sides of the ulcer with the forefinger and thumb and press for a few seconds.
4. Collect the exudates with swab stick by rolling over the ulcer, the most productive area being the moist area just inside the ulcer rim.
5. If there are more than one ulcers, then collect swabs from each ulcer.
6. Break the stem of the swab and keep it in the pre-labeled sterile screw-capped swab container. The length of the stem should be such that it fits into the container. Screw the container tightly. (In case of more than one ulcer, all the swab sticks from different ulcers will go into one container only i.e. one participant could have more than one swab sticks but only one container.)
7. Keep the container in the small zip-lock bag and zip it.
8. Place this zip-lock bag in cold thermocol box with gel packs.

9. Log each ulcer swab sample into the form and indicate ulcer location in the laboratory submission form.

If there are no ulcers reported, fill in "nil" in the ulcer location in the laboratory submission form.

D) Collection of Dry Blood Spot

Materials required:

1.Disposable Gloves

2. DBS paper with five circles (No. 903, Schleicher and Schuell)

3. Alcohol swabs

4. Lancet

5. Tissue paper

6. Preprinted label

Procedure for collection of blood for DBS:

1. For each subject two DBS papers will be used. The patient will be pricked twice and five blood spots will be collected from each prick on two DBS papers.
2. Stick the label with the participants study number on the filter paper card.
3. Select two ring fingers of each hand.
4. Thoroughly cleanse the finger with alcohol swab. Allow to air dry a few seconds.
5. Use a sterile, disposable lancet to puncture the skin off to the side of the finger tip.
6. Wipe away the first small drop of blood with a tissue paper.
7. Place the card close to the lanced area but do not touch it. Apply gentle pressure to the base of the finger and allow the second LARGE blood drop to fall from the tip of the finger onto surface of the filter paper.
8. The filter paper cards come with printed circles, apply blood to the inside of each of the circles. Attempt to fill the circle completely with a single drop before moving to the next empty circle.
9. Apply blood to only one side of the filter paper (the side with printing).
10. When all circles are filled (or client no longer bleeds) apply alcohol swab to the puncture site until blood flow stops.
11. Avoid touching the part of the card with the blood spot
12. Dry all specimens at least 3 hours in a suspended horizontal position. Depending on the climate it might be necessary to allow spots to dry over night. For drying the DBS card,

place the DBS cards on the edge of the table so that the blood spots are suspended free in the air (but not hanging down) and the DBS card secured with the help of paper weight.

Please refer to diagram given below (Simple Spot Check) for checking out the correct collection of DBS.

For IDU participants who refuse a finger prick

For the participants who refuse a finger prick for DBS, a choice of collecting venous blood by needle and syringe can be given. The procedure is as follows:

1. Select the site for venipuncture.
2. Apply tourniquet a hand's breadth above the elbow. (*Caution*: Do not allow the tourniquet to remain in place for more than one minute.)
3. Instruct the patient to clench the hand to make a fist and unclench – to repeat this thrice so that the vein stands out.
4. Wear gloves.
5. Inspect the bend of the elbow to locate a suitable vein. Palpate the vein with the finger tip(s) to determine the direction of the vein, to estimate its size and depth, and any tendency to roll.
6. Cleanse the skin of the puncture site using an alcohol swab. Allow alcohol to dry. Do not palpate venipuncture site after cleansing.
7. Remove the needle shield. Perform venipuncture with the arm in a downward position.
8. Withdraw 1ml blood with syringe.
9. After withdrawing 1ml blood place an alcohol swab over the puncture site and withdraw the needle from the vein. Do not press down on the needle. Instruct the participant to press the cotton ball over the wound for three to five minutes with the forearm folded upwards.
10. The filter paper cards come with printed circles, apply blood to the inside of each of the circles. Attempt to fill the circle completely with a single drop before moving to the next empty circle.
11. Apply blood to only one side of the filter paper (the side with printing).
12. When all circles are filled, destroy the needle in needle/syringe destroyer and discard the needle in sharps disposal container and the syringe into autoclavable bag.
13. Avoid touching the part of the card with the blood spot

14. Dry all specimens at least 3 hours in a suspended horizontal position. Depending on the climate it might be necessary to allow spots to dry over night. For drying the DBS card, place the DBS cards on the edge of the table so that the blood spots are suspended free in the air (but not hanging down) and the DBS card secured with the help of paper weight.

Packaging the DBS

Materials required:

1. Glassine paper envelopes
2. Low gas permeable ziplock bags
3. 5-10 desiccant packs
4. Humidity indicator card

Procedure for Packaging the DBS Paper:

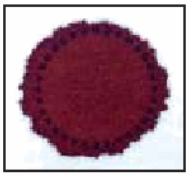
1. Once DBS are completely dry, insert one card each into one glassine paper envelope so that blood spot cards from different patients are not touching each other.
2. Pack 10-15 blood spot cards in Low Gas Permeable zip-lock bags. The following items should also be placed in this bag:
   - 5-10 desiccant packs (this will remove any residual moisture form the cards)
   - Humidity indicator cards (this will tell you the relative humidity inside the bag)
3. Press as much air out of the bag as possible and seal it shut. The humidity indicator cards and desiccant packs have a color indicator which changes from blue to pink as humidity increases. All cards and packs should be replaced with fresh material before they have all changed to a pink color.
4. Place these zip lock bags (having DBS between glassine envelope, humidity card indicator, dessicant) in the cold thermocol transportation box. *(DBS should only be taken out of cold storage when they are needed for testing)*

Diagram: Simple Spot Check

Schleicher & Schuell BioScience

Simple Spot Check

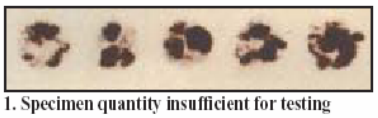
Valid Specimen



Allow a sufficient quantity of blood to soak through to completely fill the pre-printed circle on the filter paper. Fill all required circles with blood. Do not layer successive drops of blood or apply blood more than once in the same collection circle. Avoid touching or smearing spots.

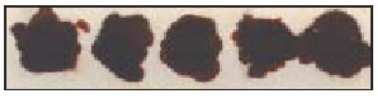
Invalid Specimens:

Possible Causes:



1. Specimen quantity insufficient for testing

- Removing filter paper before blood has completely filled circle or before blood has soaked through to second side.
- Applying blood to filter paper with a capillary tube.
- Touching filter paper before or after blood specimen collection with gloved or ungloved hands, hand lotion, etc.
- Allowing filter paper to come in contact with gloved or ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection.



2. Specimen appears scratched or abraded.

- Applying blood with a capillary tube or other device.

3. Specimen not dry before mailing.

- Mailing specimen before drying for a minimum of four hours.

4. Specimen appears supersaturated.

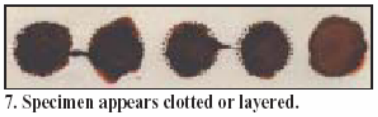
- Applying excess blood to filter paper, usually with a device.
- Applying blood to both sides of filter paper.

5. Specimen appears diluted, discolored or contaminated.

- Squeezing or "milking" of area surrounding the puncture site.
- Allowing filter paper to come in contact with gloved or ungloved hands or substances such as alcohol, formula, antiseptic solutions, water, hand lotion or powder, etc., either before or after blood specimen collection.
- Exposing blood spots to direct heat.

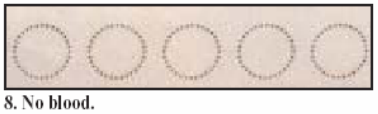
6. Specimen exhibits serum rings.

- Not wiping alcohol from puncture site before making skin puncture.
- Allowing filter paper to come in contact with alcohol, hand lotion, etc.
- Squeezing area surrounding puncture site excessively.
- Drying specimen improperly.
- Applying blood to filter paper with a capillary tube.



7. Specimen appears clotted or layered.

- Touching the same circle on filter paper to blood drop several times.
- Filling circle on both sides of filter paper.



8. No blood.

- Failure to obtain blood specimen.

Information provided by The New York State Department of Health.

Schleicher & Schuell BioScience, Inc. • 10 Optical Avenue • Keene N.H. 03431 USA • Tel. (603) 352-3810 • Fax (603) 355-6524 • solutions@schleicher-schuell.com
Schleicher & Schuell BioScience GmbH • P.O. Box 1160, D-37582 Dassel • Germany • Tel. 49-5561-791-676 • Fax 49-5561-791-583 • salesbio@schleicher-schuell.de

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Instructions to the patients after specimen collection

It needs to be clarified to the participants that the result for syphilis test will be available after ---- - (no.) days from ---- (name of) location. Also, treatment is available at ---- (name of) location and if it is positive an injection would be required.

Before the participants leaves the premises it needs to be ensured that

- All their doubts pertaining to the study or to the tests or survey have been clarified
- They have collected information pertaining to HIV/AIDS and STIs
- They have understood where to collect the syphilis test results from
- If they have requested for HIV/AIDS test they have been referred to the nearest VCTC
- They have collected the tokens where applicable.

POST EXPOSURE PROPHYLAXIS

If there is a needle-stick injury or if there is contact with any body fluid please contact your doctor as an emergency. Wash the surface with soap and water as soon as possible and follow the advice of the doctor. Post-exposure prophylaxis would require testing of both the participant as well as the lab technician.

Chapter 5

Filling of form for transporting the specimens to the district laboratory

After collecting the specimens from each patient a form (pl. see next page) needs to be filled.

Instructions for filling the lab submission form:

1. Fill one form for each day and write the date on the top where indicated.
2. The form should be filled in duplicate– one copy to go with the specimens and one copy to be maintained with the LT. (The lower half of the copy that goes to the district, will be filled by the district lab and returned to the field. This will let the field team know that the specimens have been received.)
3. Page no. will be 1. If specimens from more than 15 participants are collected on one day then use a separate sheet. The page number of this second sheet will be 2. The cluster no. etc will be given by the supervisor.
4. Time of collection of specimens is important. Please fill in each entry for each participant as soon as you finish collecting specimen from her/him.
5. Do not leave any column blank.
6. Write the ID number of all the participants whose samples have been enclosed with the box on that day.
7. If blood sample has been collected indicate if the volume is adequate or not. If blood sample has not been collected, put a cross in the cell.
8. If urine sample has been collected indicate if the volume is adequate or not. If urine sample has not been collected, put a cross in the cell and fill the remarks column for reasons.
9. If ulcer swab has been collected indicate the location of the ulcer and if ulcer swab has not been collected, put a cross in the cell.
10. The completed lab submission form should be signed by the lab technician, supervisor and the medical officer at the end of the day before sending the specimens to the district lab.
11. Finally the form should be placed in a large zip-lock bag and packed inside one of the cool boxes to be sent along with the samples.

At the district laboratory the form is received by the laboratory technician. S/he checks the specimens for number, volume, temperature, etc. and signs the form to indicate that the specimens have been received. The bottom part of the form will be signed and given back to the person who brought the specimens and would thus be sent back to the field. This way the field team would know that their specimens have been received in good condition. The top part of the form will be kept with the district laboratory for their records.

The copies at the field level will ultimately be sent to the state laboratory. This would help in cross-checking at a later date, if required.

FORM FOR TRANSPORTING THE SPECIMENS COLLECTED IN THE FIELD

Lab submission form

State _____ District _____ Cluster No. _____

Date of collection_____

FOR USE IN FIELD	FOR USE IN THE DISTRICT LAB
Total number of participants whose samples have been collected: Total number of blood samples with this box: Total number of urine samples with this box : Total number of swabs with this box : Total number of waste disposal bags + sharp disposal container with this delivery: Lab technician's name and signature: Supervisor's name and signature: (*should ensure that all specimens from all participants have been collected)* Medical Officer's name and signature:	Date of receipt: Time of receipt: Name & Sign of person who received:

S. No.	Participant's number	Time of collection	Blood vol (adequate/ not adequate)	DBS	Urine vol (adequate/ not adequate)	Ulcer location	Remarks if any	Temp at receipt	Remarks if any
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									

Chapter 6

Packaging of the specimens

The collected samples (blood, DBS, USTT, ulcer swabs) should be transported at the end of each day from the clinic to the district laboratory for immediate processing or overnight storage in a refrigerator.

It is important that the specimens are kept close to 4^0 C. Therefore, they should be packed in the zip lock bags and kept in the cool box along with the gel packs at the earliest. It should be ensured that the gel packs are frozen. For this they require to be in the freezer for a minimum of 6 hours. Management of gel packs including ensuring supply of frozen gel packs from the district lab is important and are the responsibility of the LT.

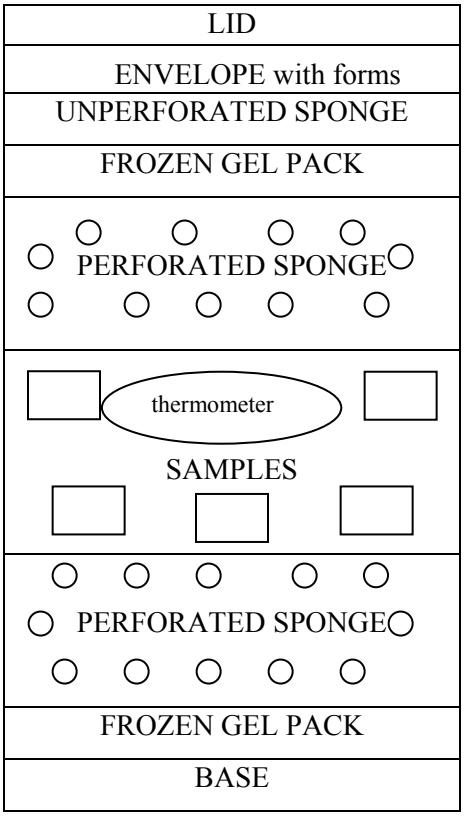
It is expected that one cool box would be able to hold specimens from roughly 5 participants. Thus, the LT should consult with the supervisor and medical officer regarding the number of participants expected and ensure adequate number of cool boxes with gel packs.

At the end of each day laboratory technician should double check all the samples for labels, before dispatching the specimen along with the laboratory submission form (filled in duplicate).

Materials required:

1. Large zip-lock bags
2. Thermocol box with frozen gel packs, sponges and a thermometer
3. Packing tape
4. Scissors

Packaging of specimens in the cool box is shown in the following schematic diagram.



The forms (laboratory submission form/ cryologs etc.) are placed in the large zip-lock bags provided for the purpose which is then placed between the unperforated sponge and the lid.

After packing the box with samples (small zip-lock bags), one thermomeeter and the forms (large zip-lock bags) seal the box lid with the packaging tape provided.

If for some reason the thermocol boxes (cool box) cannot be sent to the district laboratory on the same day, then an alternative freezer should be arranged for locally for freezing more gel packs. The maximum time that the gel packs stay frozen is about 24 hours.

If the gel packs are found to be leaking for some reason, then these can be wrapped in a plastic sheet or put into a plastic bag and used. At the end of the day's work these leaking bags should be re-checked to ensure they are still frozen.

Chapter 7

Disposal of waste in the field

Materials required:

1. Autoclavable bags
2. Black bags
3. Sharp disposal container

Disposal of sharp waste

1. All the sharp waste, like needles, should be discarded in sharps disposal container.
2. After the day's activity place the sharps disposal container in the autoclave bag and tie the bag.
3. The autoclave bag should then be transported to the district laboratory for further disposal.

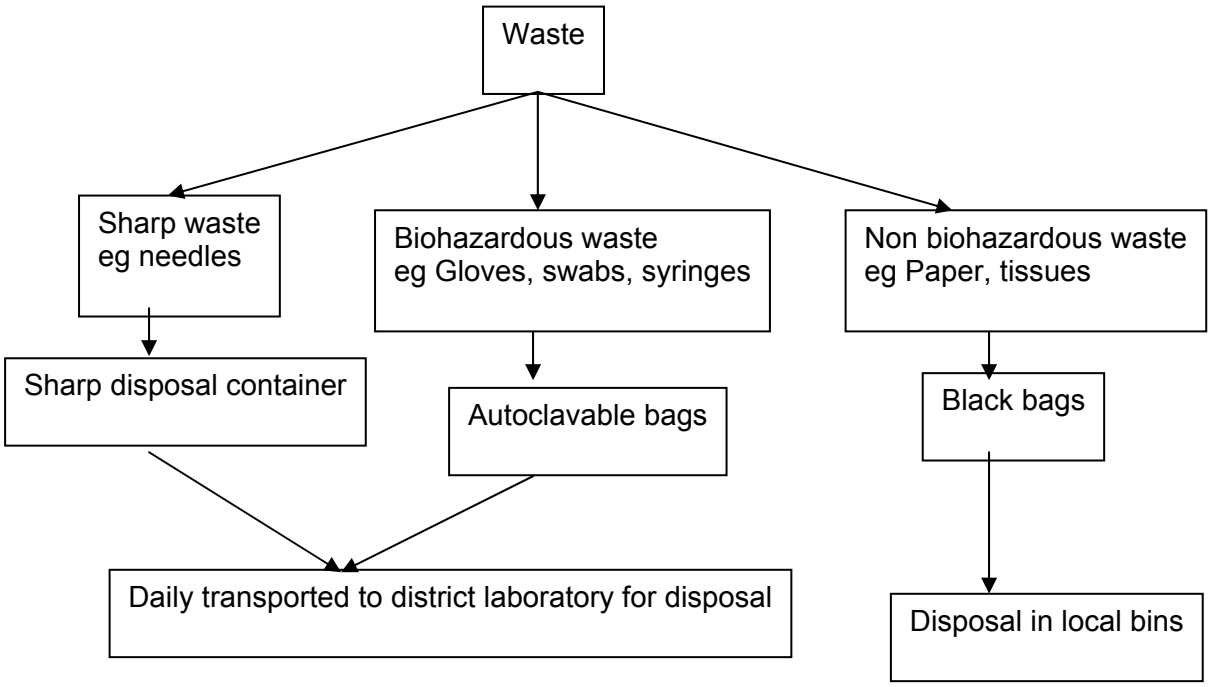
Disposal of non-sharp waste

1. All the biohazardous waste, except the sharp waste, like gloves, syringes etc should be discarded directly into another autoclavable bag (not the same as the autoclave bag containing the sharp wastes).
2. After the days activity, these autoclavable bags can double packed and tied. Tie tightly so that there is no spill but do not tie such that it is impossible to untie.
3. These autoclavable bags should be transported to the district laboratory for further disposal.

Disposal of non-biohazardous waste

Stationery and the other non-biohazardous waste should be discarded in the black bags and discarded into local disposal bins.

FLOW CHART FOR WASTE DISPOSAL



If the laboratory technician faces any difficulty with any activity in the field, s/he should unhesitatingly contact the doctor or the supervisor of the team.