Integrated Biological and Behavioral Assessment (IBBA) in Tamil Nadu, Andhra Pradesh, Maharashtra, Manipur and Nagaland, India

MANUAL FOR DISTRICT LABORATORY STAFF

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

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CHAPTER 1

INTRODUCTION

The purpose of the Integrated Bio-Behavioral 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 survey. Details of the survey and its behavioral component are given elsewhere.

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

An introduction to activities in the field

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 field laboratory technician

- Ensuring adequate stock and maintenance of all consumables and non-consumables required for the biological component of the survey
- Correct labeling 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
- Coordinating and assisting as a team member

Biological specimens to be collected from the study participants

- Urine
- Blood (clotted or dried blood spots)
- Genital Swab

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 positive samples	State
3	Syphilis	ELISA for antibody to TP (Treponistica)	DBS	All DBS samples	State
4	HIV (Human Immuno-deficiency virus) (prevalence)	ELISA (twice)	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
8	HBV	HBsAg (ELISA)	DBS	All DBS samples	RMRC
9	HCV	Screening test & RIBA (confirmatory test)	DBS	All DBS samples	RMRC
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 population, the Dried Blood Spots (DBS) 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 specimens to be collected from the participants are as follows:

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

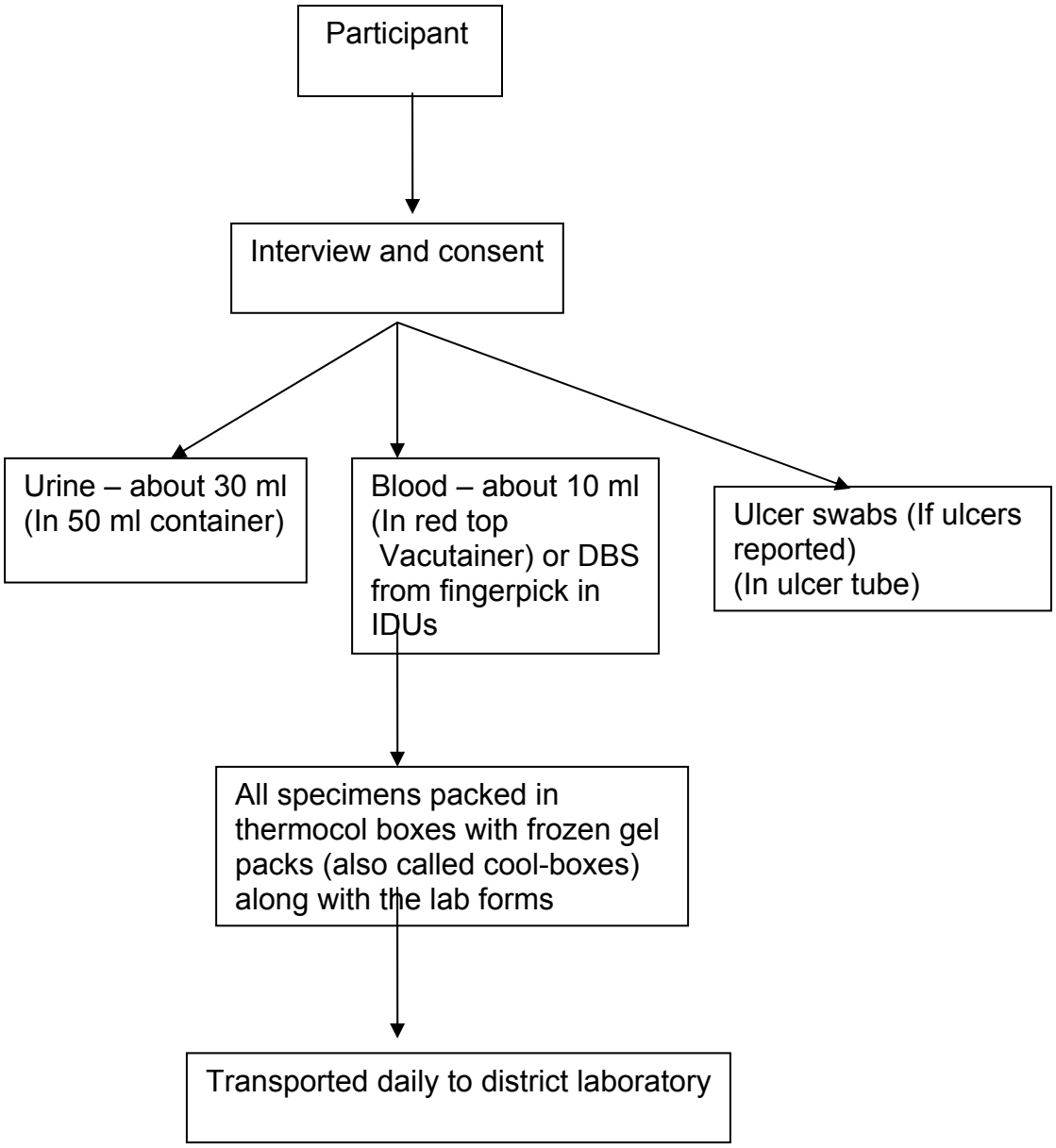
The specimens collected from the field will be transported to the district, on to the state and then on to the national level. During the transportation appropriate conditions would be maintained to preserve the specimens, to avoid contamination and avoid errors in recording and reporting. Also, each 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 – this will be at each state head quarter
3. District level

FLOW CHART FOR COLLECTION OF SPECIMENS



CHAPTER 2

Activities at the district level laboratory

The specimens will be transported daily from the field to the district level laboratory. The blood specimen will be subjected to RPR test for syphilis. This is the only test being conducted at the district level. The other tests on blood and the other specimens will be done at the state and national level (see table in chapter 1). The other important activities at district level are to aliquot the samples for testing before transportation to higher levels, maintaining the temperature of the specimens and the aliquots and transporting the aliquots to the higher levels, freezing the gel packs and sending them to the field level team. In summary, following is the list of activities at the district laboratory:

A) Receiving of all types of specimens from the field
B) Freezing the gel packs for the cool box for the field
C) Sorting of specimens
D) Ensuring right temperatures
E) Processing of specimens
F) Packaging and Transportation of specimens (aliquots) to the state laboratory.
G) Safe Disposal of waste generated from field and district lab
H) Conducting the RPR test on serum samples

The staff at the district laboratory

The staffing given here is to be considered as a model and could be different in different laboratories.

Staff required in the district level:

1. Senior LT
2. Junior LT – evening shift
3. Junior LT – morning shift
4. Cleaner
5. Night watchman

Job responsibilities of Senior LT

1. Overseeing aliquoting of serum specimen and proper storage of all samples
2. Performing RPR
3. Preparing RPR reports for dispatch to field
4. Maintaining records
5. Maintaining stores – indenting from state & issuing to field
6. In charge of administrative issues including accounts
7. Training of field LT & Jr. LTs A&B
8. Supervising Junior LTs
9. Substitute for Junior LTs as required (2 days/week when Jr LT B is traveling to state lab.)

Working hours – 9 to 5 pm (approximately)

Job responsibilities of Junior LT - evening shift

1. Receive boxes with specimens from the field
2. Check for temp, volume and labeling of specimens
3. Endorse specimen receiving forms
4. Stack the samples & gel packs in correct storage locations
5. Separate, prepare 3 aliquots of serum & store as soon as they are received
6. Assist Senior LT in maintaining stores
7. Preparing boxes & field supplies for the next morning
8. Monitoring evening temperatures on refrigerators & freezers
9. Operating generator in the evening shift – stacking gel packs around samples if power failure > 1h
10. Autoclaving all bio-hazardous wastes from field and district

Job responsibilities of Jr. LT - morning shift

1. Dusting & cleaning all equipment including biosafety cabinet, UV light & laminar flows
2. Assist Sr. LT in performing RPR test
3. Packaging all samples for transportation to state including filling of submission forms
4. Monday, Thursday travel to state lab with samples after the driver from field has left
5. Monitoring morning temperatures on refrigerators & freezers
6. Operating generator in the morning shift – stacking gel packs around samples if power failure > 1h
7. Ensure cleaner removes all disposed waste
8. Giving over to evening shift Jr. LT

Job responsibilities of laboratory attendant

1. Dusting and cleaning furniture
2. Sweeping & swabbing lab floors
3. Removing all wastes including bio-hazardous autoclaved waste and dumping it in the local municipal bin

Job responsibilities of night watchman

1. Operate generator from 9.30 pm to 8 am - stacking gel packs around samples if power failure > 1h
2. Security

The minimum equipment expected to carry out these functions in the district laboratory is

Equipment

1. +4.C Cabinet
2. Freezer of -20 deg C
3. RPR rotator
4. Bio-safety cabinet
5. Generator back up (5 KVA) if necessary
6. Autoclave for waste disposal
7. Serum separation centrifuge
8. Micropipette
9. Small oven in sites collecting DBS

Space

1 room (lab) + 1 store

CHAPTER 3
RECEIVING SPECIMENS

All the specimens from the field will come to the district laboratory at the end of the day. District laboratories would receive following specimens and materials from the field:

- **Urine Specimen transportation tube (USTT).**
- **Ulcer swabs in tubes**
- **Blood in vacutainer/DBS in zip lock bags.**
- **Waste bags (autoclavable or disposable) and sharp disposal containers**

Each lot of specimens will be accompanied with two copies of a form signed by the lab technician, field supervisor and the medical officer in the field. The original copy of this form would have been kept by the field level laboratory technician (LT). This would help her/him in tracking the specimens sent to the district laboratory on a particular day.

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

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 (30ml) Yes/No	USTT	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										

A designated technician will be in charge for receiving the specimen. All the received specimens will be checked for volumes, temperatures, leakages and labeling of the specimens. All the specimens received should be logged in specimen submission form received from the field. One of the signed copies of this form would be kept with the district lab for records and the other signed copy should be sent back to the field LT as evidence that the specimens have been received in the recommended conditions. For instance if the specimens were not received in the recommended temperature range, then corrective actions need to be taken by the field level team.

CHAPTER 4
SORTING AND PROCESSING OF SPECIMENS RECEIVED FROM THE FIELD

The specimens are expected to be received late in the evening in the district laboratory. It is important that the blood samples are processed and sorted out in the evening itself. The urine specimens however, can be processed the next morning. (This is one of the reasons for the district laboratory personnel to work in shifts.)

SORTING OF SPECIMENS

To streamline the laboratory activities it is important to mark space for storage of vacutainers, for storage of urine specimens and for storage of ulcer swabs. The ulcer swabs are going to be fewer in number compared to the other specimens. (all the participants in the study might give blood and urine samples but swabs are only from participants who report an ulcer which is roughly 10-15%.). Thus, while sorting the specimens -

1. Vacutainers/DBS cards will be placed in the racks and placed in the refrigerator rack designated for blood
2. Urine in specimen transportation tube will be placed in the refrigerator rack designated for urine
3. Ulcer swabs will be placed in the refrigerator rack designated for ulcer swabs

Please note that the specimens are not to be stored in the freezer at the district level.

SPECIMEN PROCESSING

1. BLOOD

This should be done as soon as the specimens are received from the field.

Purpose:

Serum will be separated from the blood and 3 aliquots will be made for the different testing on the serum.

One aliquot will be used for RPR testing at the district laboratory and remainder 2 aliquots will be sent to state laboratory. Remainder serum from vial on which RPR test is done will also be sent to state lab for TPHA testing (positives on RPR) or storage (negative on RPR)

Materials required:

1. Centrifuge
2. Five cryo storage vials
3. Sterile Pasteur pipette
4. Gloves
5. Preprinted labels with the specific ID number of the study participant (5)
6. Cryo storage box

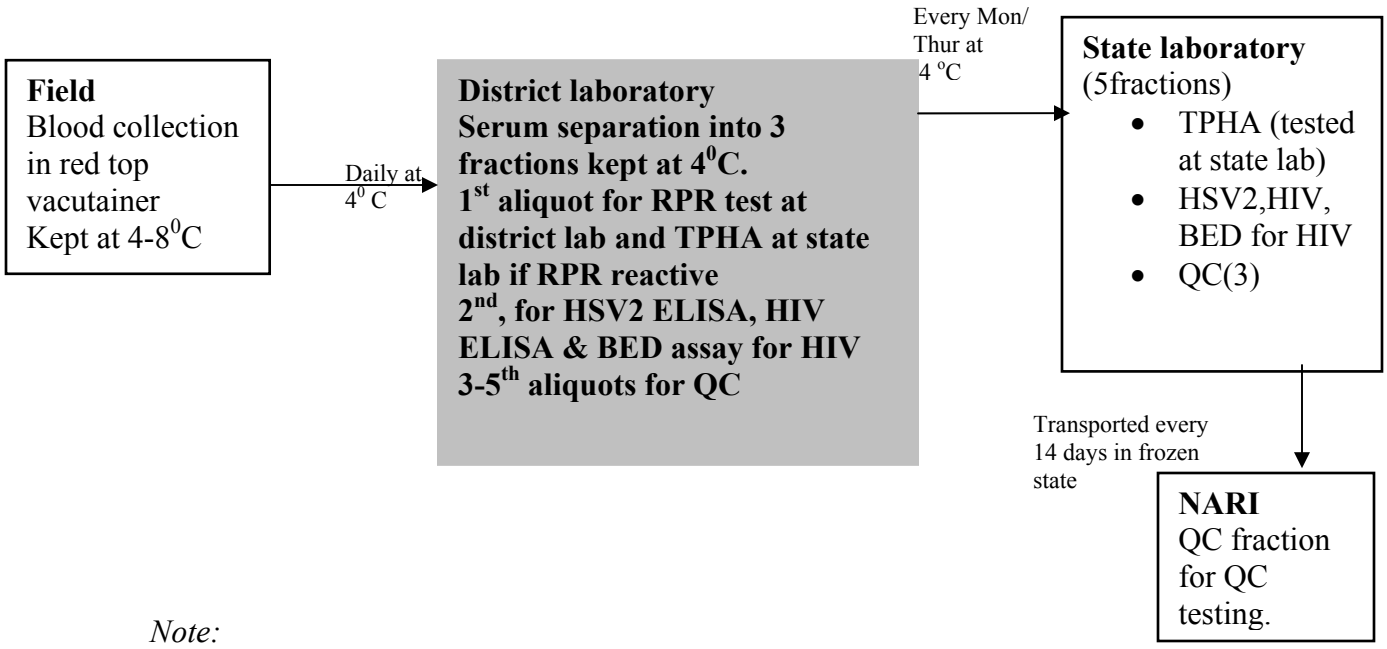
Processing in the Laboratory:

1. To process the whole blood (approx 10 ml), the blood tube should be centrifuged at 1000 rpm for 10 minutes to sediment the cellular material. In these red top tubes you will see serum at the top most layer as clear straw colored layer and a layer of clotted RBC at the bottom of the tube. Separate the serum present in the upper part of the red top tube by using a disposable Pasteur pipette into aliquots as mentioned below.
2. The serum fraction should be split equally into three parts using sterile disposable Pasteur pipettes. About 1.5 ml each should be separated into three cryo storage vials for:
   i. One tube for RPR (district lab) and TPHA (state lab if RPR is reactive)
   ii. One tube for HIV ELISA, BED Assay, HSV2 ELISA.
   iii. Three tubes for QC/Long term Storage
3. Each of the tubes should be pasted with one pre-printed label giving the ID number of the study participant and the purpose for which the serum will be used. The LT should ensure that the number on the vacutainer tube matches the number on the aliquot tubes. These tubes will have been labeled with the three pre printed labels as e.g.,

1234567 Serum: RPR and TPHA	1234567 Serum: HIV and HSV2	1234567 Serum: QC

4. The tube should not be filled more than ¾ full to prevent the top coming off when the serum is frozen.
5. The cryovials will be separated into their respective cryo-storage boxes- One box for HIV tests, BED assay for HIV, HSV2 tests and one box for QC. Those serum which will be positives on RPR will be placed in 1 cryostorage box and those which are negative will be placed in other cryostorage box. Cryo storage logs will be maintained as in appendix E. Cryostorage box will be stored at 4^0C till transported.

FLOW CHART FOR BLOOD HANDLING



Note:

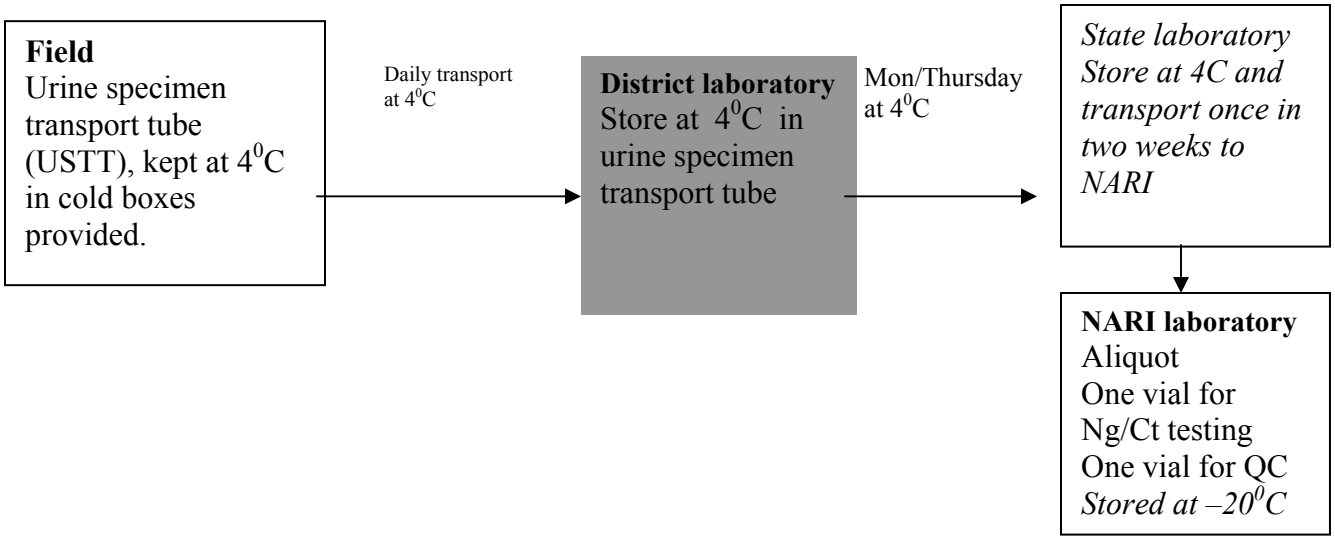
- *The laboratory technician should wear gloves all the times while handling the specimens.*
- *Waste generated should be disposed as in Appendix C*

2.URINE SPECIMEN TRANSPORTATION TUBE (USTT)

Purpose:

- The urine specimen transport tube from field the would be stored at 4˚C in the refrigerator. The ziplock bag containing the urine transportation tube will not be opened and the tube will be stored as such in the refrigerator.

FLOW CHART FOR URINE HANDLING



3. ULCER SWABS IN SWAB CONTAINER

Purpose

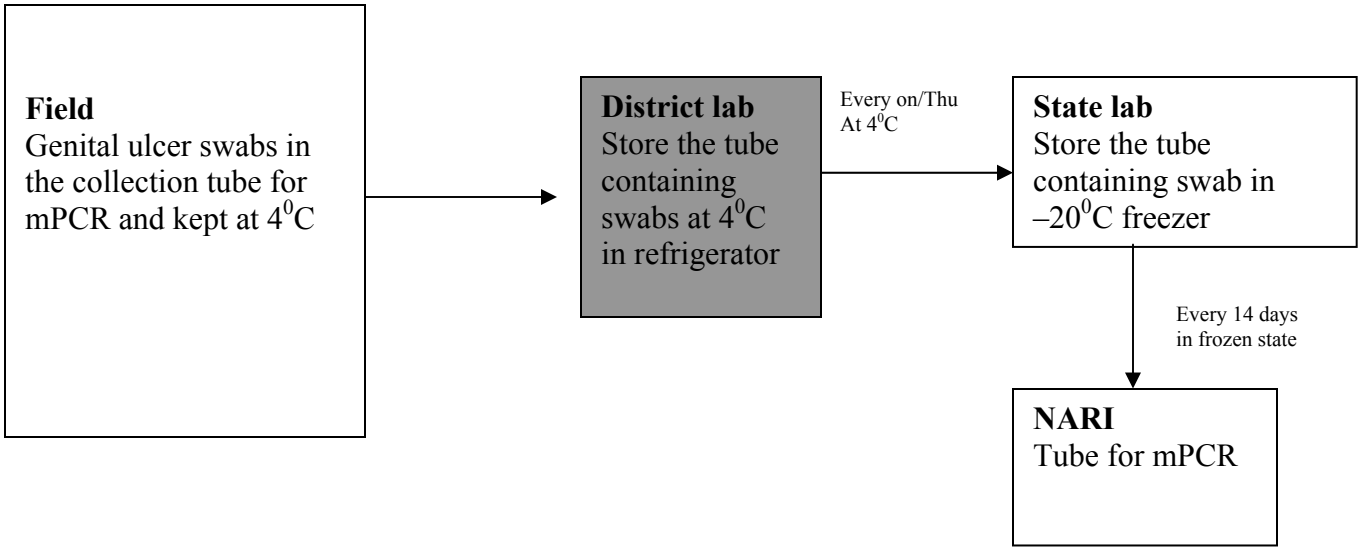
For mPCR to be performed at NARI

Processing at the laboratory

No processing at the laboratory.

Only storage at 4^0C without opening the zip lock bag, later to be transported to district laboratory in the cold thermocol transportation box.

FLOW CHART FOR ULCER SWAB HANDLING



4. DBS PAPER

Purpose

For HSV2 ELISA, HIV ELISA, BED assay for HIV and TP ELISA testing.

Processing at the laboratory

No processing at the laboratory.

Place the Gas impermeable plastic bags with 20 desiccant pouches and humidity indicator card in the air-tight plastic container in the refrigerator at 4 ^{0}C. Storage at 4^{0}C, later to be transported to district laboratory in the cold thermocol transportation box. The humidity indicator card should be checked daily and if the humidity increases more than 30% the desiccant pouches should be changed. The desiccant pouches should be dried by placing inside a oven at 45 deg C for one hour.

5. WASTE BAGS AND CONTAINERS RECEIVED FROM THE FIELD

It is important that the bio-hazardous wastes generated as a result of IBBA are taken care of appropriately. Since the resources at the field are limited and also because the field sites might not be well laid out sites, the district team is given this responsibility. The procedure for waste disposal ensures maximum safety for the staff handling the wastes.

Processing

The wastes from the field will be received in appropriate sharp containers, and autoclavable bags. The non-biohazard us waste will be in disposal bags. The field has been provided with needle destroyer and the needles will be destroyed before being packed in sharp disposal container.

Sharp disposal containers and the bags (autoclavable and disposable) should be autoclaved at the district laboratory and then discarded into municipal bin. The sharps should be transferred to card board box and then discarded.

The recommended practices for operating autoclaving are -

1. Place the autoclave bag with the waste in the autoclave and the sharps disposal containers.
2. Loosen the lid and the cap of the sharps disposal container.
3. Close the autoclave and switch it on
4. Let the pressure reach 15 psi on the gauge (at which point the temperature inside the autoclave would be 121^{o} C)
5. Keep the autoclave on for 15 minutes after the pressure has reached 15 psi
6. After switching off the autoclave allow it cool for ½ hour before opening

CHAPTER 5
SPECIMEN STORAGE

All the specimens should be stored in the 4°C cabinet, until transported every Monday/Thursday to State laboratory.

DBS Card Storage:

1. Check the color of the humidity indicator card. All cards and desiccant packs should be replaced with fresh material at 30% of humidity (when the humidity indicator card circle for 30% turns pink).
2. Desiccant packs should be warmed for 3-4 hours at 45 deg C before use.

For short term storage, DBS should be kept in zip-lock bags with desiccant stacked in a plastic container and stored at 4°C. DBS should only be taken out of cold storage when they are needed for testing.

Typically following samples would be stored from each patient:

i. Serum: 5 serum vials
ii. Urine: Urine specimen transportation tube
iii. Ulcer swabs: 1,2,3---

Organize the racks of 4^0 C refrigerator so as to arrange the different shelf for various samples:

Rack 1 for serum/DBS cards
Rack 2 for urine
Rack 3 for ulcer swabs

Guidelines for storage of specimens

- Store specimens in the refrigerator at the temperature of 2-8°C. Designate each rack for each type of specimen e.g. all urine specimens in first shelf, all blood specimens in the second shelf and all swabs in the third shelf.
- Check temperature of the refrigerator every day and maintain a temperature log as shown in this table. (Appendix I)

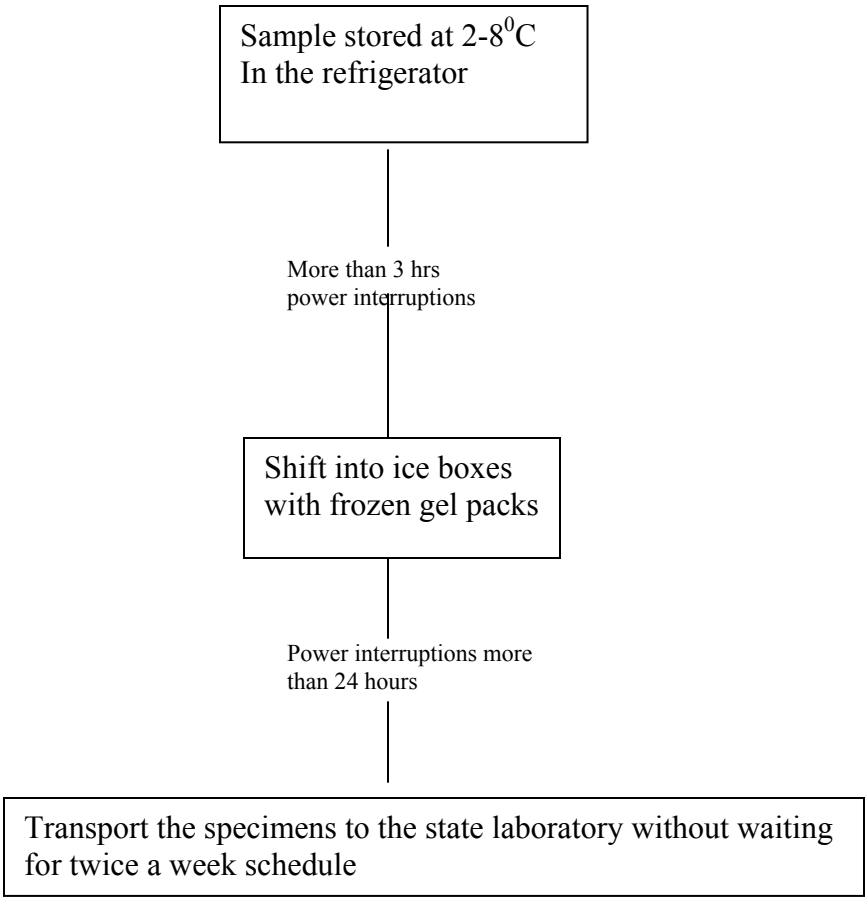
If there is power interruption:

Back up generator (if available) should be immediately turned on.

In cases of non availability of generator:

1. Specimens stored in refrigerator at 2-8^0 C: After 1 hr of power interruption shift them to boxes filled with frozen gel packs. Can be stored in this condition for up to 24 hrs.
2. If power interruption lasts for more than one day, transport the specimens to the state laboratory, without waiting for twice every week cycle.

FLOW CHART FOR MANAGING SPECIMENS IN CASE OF POWER FAILURE



CHAPTER 6
LABORATORY TESTING

SYPHILIS TESTING

Specimen Processing and testing for syphilis

1. One of the serum tubes will be examined for RPR at district laboratory.
2. The RPR test will be set up everyday from Monday-Friday at the district laboratory.
3. All reactive RPR will be examined quantitatively at the district laboratory and confirmed with TPHA at state laboratory.
4. All the reactive specimens will be transported to state laboratory twice every week, Mon & Thur at 4^0C for TPHA testing.

RPR TESTING

Laboratory Requirements:

- Mechanical Rotator, circumscribing a circle of 2 cm in diameter at a speed of 100 rev per minute
- Timer
- Light source for reading results.
- Micropipette
- Gloves.
- Marker

Reagents

RPR antigen test (SPAN Diagnostics)

INTENDED USE

Rapid test for the qualitative and quantitative detection of syphilis in serum or plasma

SUMMARY AND TEST PRINCIPLE

The RPR test is a nontreponemal test for the rapid detection of syphilis. The RPR antigen suspension is a variation of the VDRL antigen containing some additives to eliminate the

need for heat inactivation of serum, to enhance the stability of the suspension and to improve the visual reading of reactions.

The RPR antigen is a cardiolipin suspension containing charcoal microparticles. This antigen detects an antibody, called "reagin", present in the serum of syphlitic patients. When sample contains reagins, a flocculation of the antigen is produced, which coagglutinate with the charcoal microparticles giving black clumps of different size depending on the reagin titer. With nonreactive samples, no reaction will take place and a homogeneous grey colour will be maintained.

Test Procedure

Before starting the testing take the kit out from the refrigerator and bring to room temperature.

Qualitative Test

1. Place one drop of serum (50µl) on the card with the help of micropipette. Set up a positive and negative control with each run.
2. After gently mixing the RPR antigen suspension, place one drop (15-20µl) using the antigen delivery dropper.
3. Mix these drops well and spread out the pool of liquid uniformly within the entire area of the circle by using the disposable applicator stick supplied in the kit.
4. Rock the card with mechanical rotator for 4 minutes and observe under good light source for appearance of agglutination.
5. Do not reuse the card.

Quantitative Test

1. Place 50µl of normal saline onto card circles 1 through 5 with the help of micropipette.
2. Using 50µl micropipette, add 50µl of the sample to saline in first circle
3. Using the same micropipette, mix the sample with saline by aspirating back and forth several times. Aspirate 50µl from the 1st circle and transfer

to 2nd circle. Repeat the same operation successively up to the 5th circle. Aspirate 50µl from the 5th circle and discard.

4. Carry out steps 2 through 4 described under qualitative test with each drop of diluted sample.
5. The end point is the highest dilution showing any visible aggregation of black particles.

III. Quality Control

1. The negative control should give a negative result.
2. The positive control should give a positive result.

IV. Interpretation of Test Results

Positive: Characteristic black aggregates, which may be deposited at the periphery of the liquid at the end of 4 minutes of rotation.

Negative: Complete absence of black aggregates with uniform grayish background at the end of 4 minutes of rotation.

V. Generation of Report:

Two types of Reports will be generate

Appendix E: This report format is to be sent back to the field –any positive titer is to be reported as positive. If no aggregates form at the end of the test it is to be reported as negative.

Appendix F::This report format is to sent to State Lab for TPHA, giving the titer as the highest dilution showing any visible aggregation of black particles.

EXTERNAL QUALITY CONTROL FOR RPR:

- All reactive RPR serum will be submitted to state laboratory for confirmation through TPHA.
- All negative serum will be submitted to state laboratory.
- 10% percent of all serum will be randomly selected and tested by RPR test at State Laboratory to validate the RPR results.

It is important to remember to save all the samples, clearly marked with ID number until the QC selection is done and store them at 4^0C until they are transported to state laboratory for QC.

References: RPR (Rapid Plasma Reagin Test) Product insert, Span Diagnostics Ltd.

CHAPTER 7
TRANSPORTATION OF SPECIMEN TO STATE

The following would be transported to state laboratory every Monday/Thursday in the cold thermocol transportation boxes.

1. Cryostorage box containing serum storage vials for HIV1&2 ELISA, BED for HIV, HSV-2 testing.
2. Cryostorage box containing serum storage vials negative on RPR for QC testing.
3. Cryostorage box containing serum storage vials positive on RPR for TPHA testing.
4. Cryostorage box containing serum storage vials for QC testing
5. Urine specimen transportation tubes for Ng/Ct Aptima Combo 2 testing in ziplock bags
6. Ulcer swab tubes in zip-lock bags.
7. DBS cards in zip lock bags.

One cryostorage box each will be placed in one gel pack box and transported every Monday & Thursday to the state laboratory. District Laboratory submission (as shown below) should be filled in duplicate will placed in large zip-lock bag and sealed and transported with the cold transportation box. One copy will be retained by State Laboratory and one copy will be received and a portion returned to District Laboratory.

For packaging and transportation of the specimens, follow the instructions given on the thermocol boxes or refer to the AppendixA

THE LAB NEEDS TO MAINTAIN A BOOK FOR THE RPR TEST (& similarly for all the other tests along the following lines)

Date –

Date	Participants' ID no.	Result of RPR test	If RPR positive, pl write the dilution	Has the test result been communicated to the field (yes/no)	Signature of LT

DISTRICT LABORATORY SUBMISSION FORM

SPECIMEN TYPE: (please encircle)

Serum RPR positive / Serum RPR negative / Serum QC / Serum HIV, HSV2 /

Urine Transportation tube / Ulcer Swabs / DBS cards

	1	2	3	4	5	6	7	8	9	10
A	PARTICIPANT ID No.									
B										
C										
D										
E										
F										
G										
H										
I										
F										

Total number of participants whose samples have been sent _____

Lab technician's name and signature: _____

Supervisor's name and signature: _____ (*should ensure that all specimens from all participants have been collected)*

(The section below has to be filled at the State lab)

To be filled at the State lab (Date____, State____)

Name, designation and signature of the person who received the specimens _____

Date and time of receipt _____

Samples were frozen : Yes/ No

Remarks if any_____

CHAPTER 8

Store and Supplies

A secure and proper room (away from heat, humidity and free of pests) should be designated as STORE for storing of the consumables for the activities to be carried out at district laboratory. The store should have a dedicate cold storage facility i.e. 4^0C Cabinet. The stocks should be on racks/ tables and not on the floor and 6 inches away from the walls. A technical person should be made in-charge of the stores. The responsibilities of the store in charge would be:

1. To indent the consumables from the state lab

2. To receive, accession and issue the consumables. Before receiving the material, it should be inspected and checked on following parameters:

    A. Transportation Conditions
    B. Quantity
    C. Quantity indent and quantity received
    D. Physical state of the item.

3. After receiving, the material should be immediately accessioned in the Stock Status Register (Appendix B). All the columns in the stock register should be duly filled. With every transaction the balance stock should be filled. If the store incharge is not competent to inspect the technical items, another technical person should be deputed for inspection of items and he should sign in the stock register.

4. The store incharge should be provided with a list of authorized person who can approve the indent form with specimen signatures. After receiving the indent form duly signed by the competent authority with official seal, the material should be issued and signature should be taken.

5. A indent form File (Appendix D) should be created to file the indent forms. The file should be divided into two parts. Part one should have indent against which the complete issuing have been made and part two should have indent against which the issuing is pending. It will help the store incharge to track the pending indent forms. The store incharge should write the contact details of the concerned person who has submitted the indent form and he should be immediately informed when the store incharge have the enough stock. It is the responsibility of the store incharge to issue the indent form number at the time of submission of indent form by the field worker and it should be conveyed to the field worker for his future reference. The field worker should take receiving of submission of indent form from store incharge.

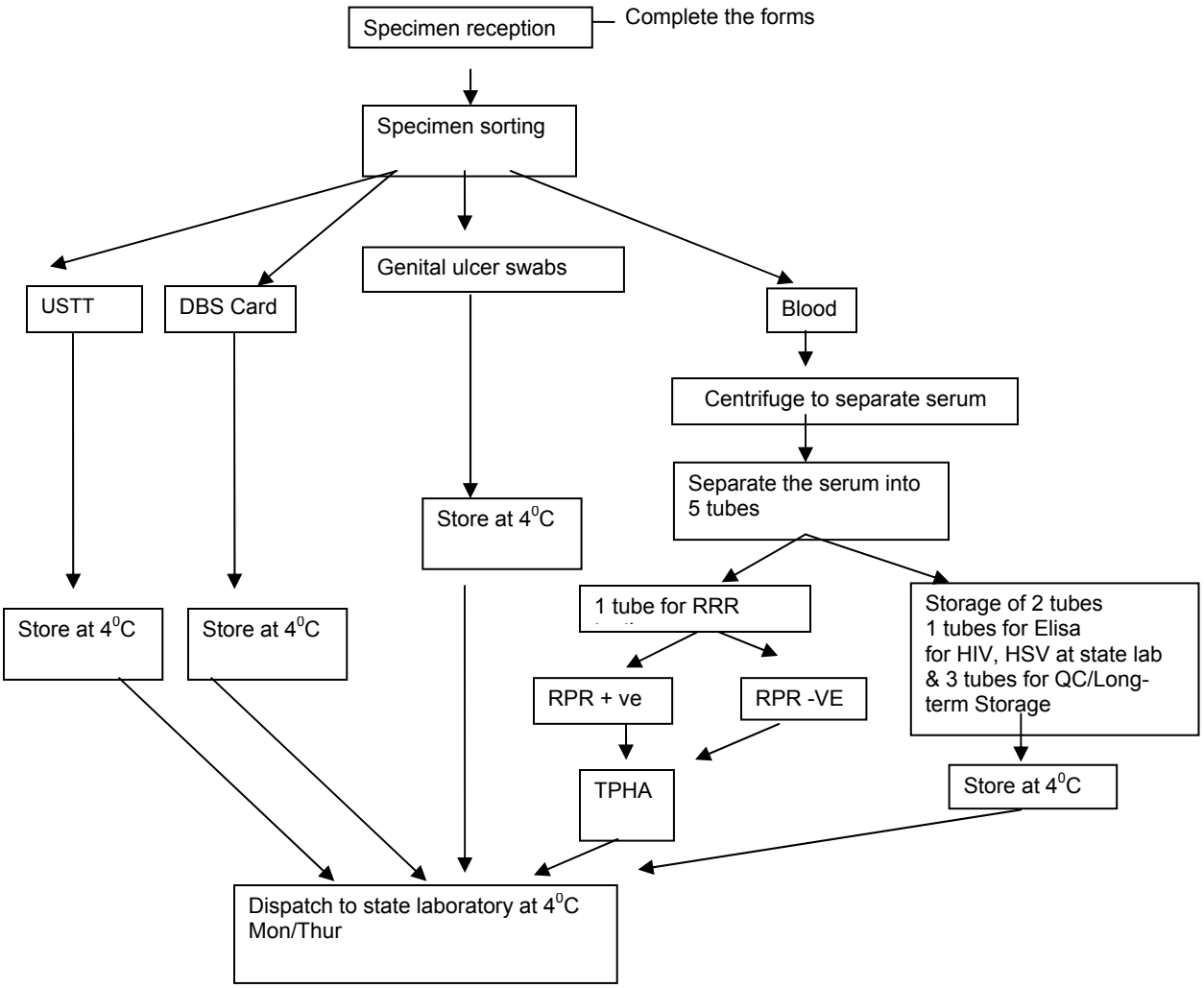
6. The store incharge is responsible for maintaining minimum stock level as per appendix-H . When the stock level reaches the re-order level or minimum stock level, the store incharge should prepare a indent form and immediately send to state store and follow it up on regular basis. A list of re-order quantity is also provided in Appendix H.

7. It is the responsibility of the store incharge to store the material at proper temperature and place.

8. Store incharge should prepare a list of locations for all items within the store i.e. like Rack no. or drawer no. etc.

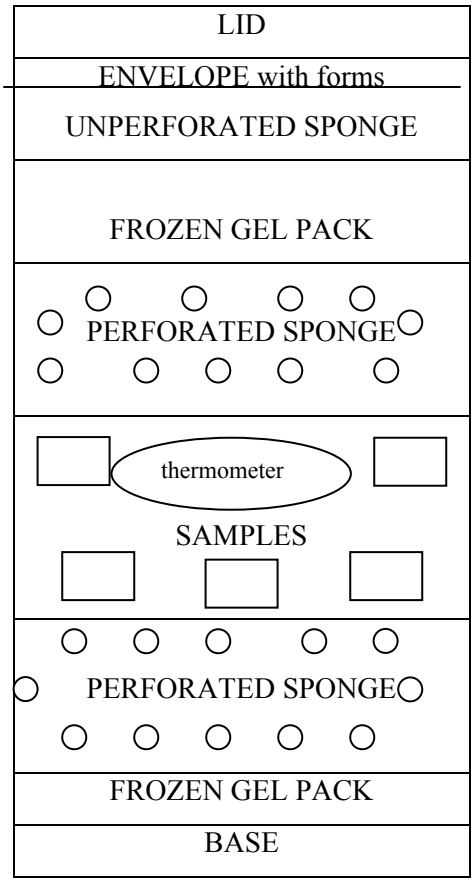
9. It is the responsibility of the store incharge to ensure at leat 25 gel packs should be kept frozen at all the times for giving it to field staff and for packaging the cryostorage box and specimens to the state laboratory.

FLOW CHART FOR COLLECTION AND PROCESSING OF SPECIMENS AT DISTRICT LABORATORY



APPENDIX A

DIAGRAMM ATIC REPRESENTATON OF SAMPLE PACKAGING INSTRUCTIONS IN THE THERMOCOL GEL PACK BOX



PACKING INSTRUCTIONS

1. Ensure that gel packs are kept for a minimum of 6 hours in the freezer so that it is completely frozen before packing
2. Screw the lid of the sample container tightly and kept in the ziplock bag
3. Ensure proper labeling of sample container
4. Please keep properly filled forms (laboratory submission form/ cryologs etc. in large ziplock bag provided and place it between unperforated sponge and lid
5. After packing seal the box lid with packaging tape provided

APPENDIX C
WASTE DISPOSAL POLICY

SHARP WASTE

The sharp containers will be received each day, in the evening, along with the specimens from the field. All these sharp containers will be autoclaved with the lid loosened. All the sharp waste will then be put into a cardboard box and discarded in local disposal bin.

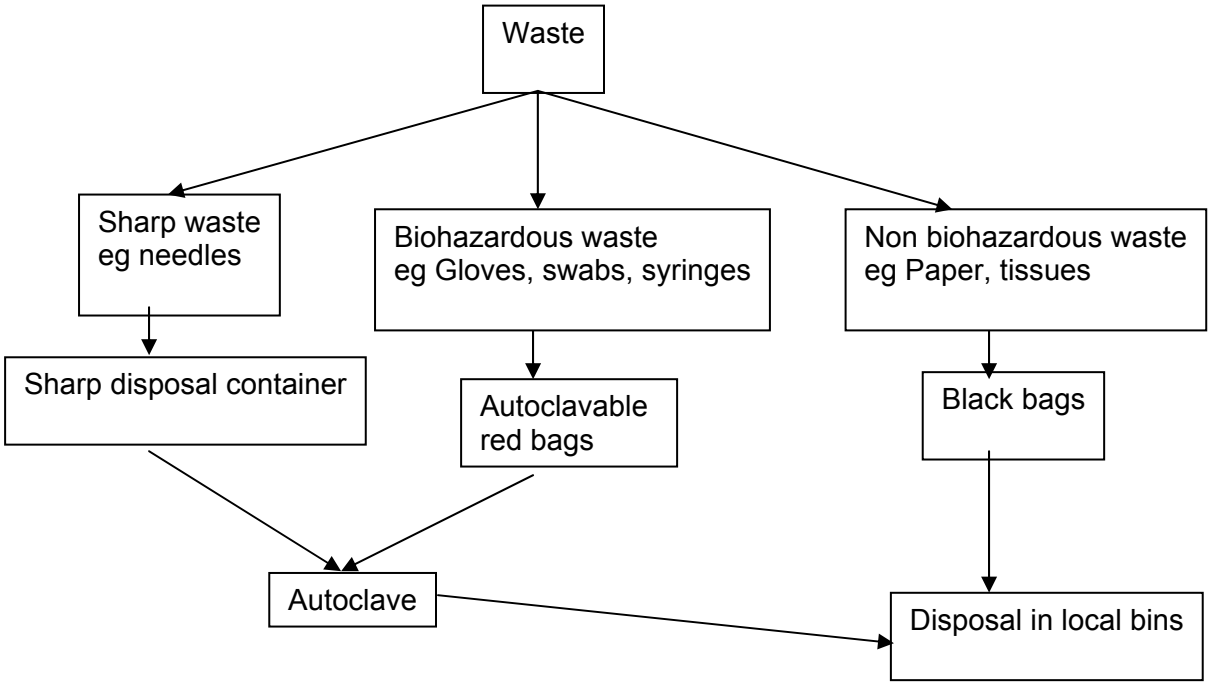
NON-SHARP BIOHAZARDOUS WASTE

1. All the biohazardous waste, except the sharp waste, like gloves, syringes etc should be discarded into autoclavable bags provided.
2. After the days activity, these autoclavable bags can be double bagged and tied to avoid leakage due to tears in the autoclavable bags.
3. These autoclavable bags then can be autoclaved and discarded into local disposal bin.

NON BIOHAZARDOUS WASTE

The black bags received from the field will contain the waste generated from the stationery etc. and these can be discarded into local bins in the field or if that is not possible they can be sent to the district. All the stationery and no biohazardous waste should be discarded in the black bags and discarded into local disposal bins.

FLOW CHART FOR WASTE DISPOSAL



Appendix E

Integrated Bio-Behavioral Assessment in India
Laboratory result form

Date of sending the result_____

Participants ID No: _____

Name of the Referral clinic: _____

Name of the field supervisor who sent the sample: _____

Specimen: Serum

Date of collection: _____ Date of test: _____

Test done: RPR

☐ Reactive

☐ Non-reactive

Laboratory supervisor: Name _____ Signature _____

Appendix F

IBBS
LABORATORY RESULT FORM (DISTRICT LABORATORY)

Participant's ID Number: _____
Study Site: _____ Signature of LT: _____

ENCIRCLE THE CORRESPONDING NUMBER

.

Blood (Tested at District laboratory)s	
Date Received:_____Date Tested:_____ Laboratory technologist: Name _____Signature_____ Supervisor: Name _____Signature_____	
a. RPR Result	0. non reactive 1. reactive 2. indeterminate
b. If reactive: Titer	1. 1:2 2. 1:4 3. 1:8 4. 1:16 5. 1:32 6. 1:64 7. 1:128 8. ABOVE

Remarks: _____
_____

Date:

Appendix H
Minimum stock maintenance and order sheet at district laboratory

	Item	Minimum stock level	Reorder Quantities
1	Vacutainers	130	260
2	Vacutainer holder	2	4
3	Alcohol swabs	130	260
4	Band aids	130	260
5	Tourniquet	2	4
6	Pre-printed label	For 130 subjects	For 130 subjects
7	Markers	5	10
8	Small zip-lock bags	500	1000
9	Gloves	1000 pairs	2000 pairs
10	Urine container	130	260
11	USTT	130	260
11	Cello tape	10 rolls	20 rolls
12	Packaging tapes	10 rolls	20 rolls
13	Gel-pack boxes	50	100
14	Large zip-lock bags	100	200
15	Dacron swab with container	50	100
16	Scissors	5	10
17	Sterile cotton balls	50	100
18	Disposable pipette	130	260
19	Cryostorage vials	1000	2000
20	Cryostorage boxes	20	40
21	Bleach solution (1 litre bottle)	10	20
22	Sterile saline 500ml bottle	2	4
23	RPR test kit	1	2
24	200 ul tips	250	500
25	Discarding beaker	5	10
26	Sharp container	2	4
27	Autoclavable bags	50	100
28	Black disposal bag	50	100

APPENDIX I
Temperature Log

Performed: Daily at beginning of shift Year: _____
°C- Celsius
Location: _____ Expected value: _____
I- Tech Initials

Date	Jan		Feb		Mar		Apr		May		June		July		Aug		Sep		Oct		Nov		Dec	
	°C	I	°C	I	°C	I	°C	I	°C	I	°C	I	°C	I	°C	I	°C	I	°C	I	°C	I	°C	I
1																								
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31																								
Reviewed																								

Appendix J

Laboratory Work Sheet for RPR Test

Date of Testing: _____
Name of the Test **Kit Lot No_____**

Number of Tests to performed ---------- **Expiry Date_____**

Kit Controls: Reactive □ Non Reactive □ Saline Control: Neg Pos

Known previous reactive patient sample □
Known previous non reactive patient sample □

S.NO	Patient name or UID No	Qualitative Result NR or R	Quantitative Result (dil)	Report NR R --dil
1	QC 1 Kit			OK
2	QC 2 Kit			OK
3	QC 3 Kit			OK
4				
5				
6				
7				
8				
9				
10				
11	Known Pos sample			OK
12	Known Negative sample			OK
13				
14				
15				
16				
17				
18				
19				

Signature of the Laboratory Technician.

Date of Reporting