Manual for the

State level laboratory

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

STATE LEVEL MANUAL FOR THE LABORATORY COMPONENT

CONTENTS

APPENDICES:

4.

ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
BMGF	Bill and Melinda Gates Foundation
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*
GUD	Genital Ulcer Disease
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
MSM	Men who have sex with men
MSW	Male Sex Worker
NACO	National AIDS Control Organization
NARI	National AIDS Research Institute
NG	*Neisseria gonorrhoeae*
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

In India, sentinel surveillance is used annually to estimate the prevalence of HIV infection in the country and to monitor trends in the epidemic. Sentinel surveillance for HIV in India began in 1994, at 55 sentinel sites, under the National AIDS Control Program-I (1992–1999). The population groups and sites for HIV sentinel surveillance are selected based on information about the risk behavior of various risk groups for HIV infection. The high-risk groups of the population include patients attending sexually transmitted disease (STD) clinics, female sex workers, injecting drug users (IDUs), and men who have sex with men (MSM); a low-risk group of the population includes women attending antenatal clinics. The rationale for selecting sentinel sites in the clinics attended by these subgroups of the population is that blood samples are collected from the people who attend the clinics for various purposes, and the samples can be tested for HIV in an unlinked anonymous manner. Since 1994, the number of sites in the sentinel surveillance system has been increasing.

Despite the HIV, STI and risk behavior surveillance activities currently underway in India, there are considerable gaps in the information available to understand both the course of the epidemic as well as the STI correlates and behavioral risks that fuel it. To measure the major outcomes and impacts of the interventions funded by the Bill & Melinda Gates Foundation (BMGF) under the Avahan India AIDS Initiative (Avahan), the existing surveillance system must be strengthened and expanded. A robust surveillance system will allow BMGF and its governmental and nongovernmental partners not only to follow key trends in HIV, STIs and risk behaviors, but also to use the data to project trends into the future.

The purpose of this 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. The proposed mapping, size estimation and integrated behavioral and biological survey (IBBS) will provide some of the key data needed to assess major outcomes and impacts of the interventions funded by BMGF. This is the first independent impact-level evaluation of this scale of targeted interventions with sex workers and clients, high risk men and IDUs on HIV/AIDS. The project will be implemented in close collaboration with National AIDS Control Organization (NACO) and State AIDS Control Societies (SACS) and will provide valuable information to feed back into and strengthen the National AIDS Control Program in India.

The first round of survey was conducted in 2005-6 and the second round will be conducted 2009.

CHAPTER 2
Activities in the State Laboratory

This manual should be used closely in conjunction with the Laboratory SOP Manual, particularly *SOP 1.1 Specimen Management and Quality Control*.

The responsibilities at the state level laboratory include the following:

A. Specimen reception
B. Specimen sorting
C. Specimen testing
D. Specimen shipping
E. Specimen storage
F. Data management
G. Study management of district level laboratories
H. Quality control oversight

A. Specimen reception

The specimens from the district laboratory will be transported to the state laboratories. A designated person will be in charge for receiving the specimens. Specimens to be received include serum, urine, genital ulcer swabs, and dried blood spots (DBS). These will be shipped in a Thermocol box.. Specimen shipments must be accompanied with a specimen transport form (See Appendix A).

Upon receipt of the Thermocol box:

- Inspection of Thermocol box (i.e. is box intact and properly sealed, is there appear to be any damage or leakage).
- Unpack Thermocol box.. Components include cold gel pack, Ziploc bag containing Laboratory Specimen Submission Form, cryostorage boxes, Ziploc bags with tubes containing USTT, Zip lock bag containing tubes with genital ulcer swabs, Ziploc bag containing DBS, and sponges. Check the temperature on receipt (thermometer in the box) **Inspect the contents to assure the integrity of the samples and Thermocol components.**
- Cold gel packs and sponges are replaced in the Thermocol box and returned to the district laboratory.
- The cryostorage boxes and Specimen Submission Form must proceed to the Specimen sorting step.
- As specimens are sorted and tested, they must be consolidated for storage at the state laboratory. Empty cryostorage boxes need to be returned to the district laboratory for future shipments.

B. Specimen sorting

The cryoboxes from the district laboratory will be labeled as follows:

- Positive RPR (for TPHA)
- Negative RPR
- Serology tests (HIV, HSV)
- QC Vials (3 per participant)
- Urine (APTIMA Combo 2)
- Ziploc bags containing the DBSs.

Steps for specimen sorting:

- The boxes are labeled to ease the process of specimens moving quickly to the proper workbench for testing.
- Upon receipt, the specimens must be verified and accounted for by the corresponding Laboratory Submission Form.
- The integrity of the specimen and tube must be checked. All specimens will be received at 2-8°C. Verify if the contents of the box are cool. Verify if the gel packs are still frozen and cold, or if they have thawed. Document this on the District Laboratory Submission Form.
- Specimen tubes will be labeled designating which test is to be performed on a particular tube. Once samples have been verified, complete the submission form acknowledging receipt, and deliver them to the testing area.
- The lower portion of the submission form is returned to the district laboratory, and upper portion will be kept by the state laboratory.
- Specimen tubes received will be:
  Serum vials [1. Syphilis, 2. HIV (plus 10% will be for HSV), 3. QC vials -3]
  Urine Sample Transport Tube (USTT)
- **NOTE:** All serum samples must be frozen within one week of collection. Samples must be stored at -20°C. It is very important to minimize freeze/thaw cycles. Plan testing accordingly.
- **NOTE:** Urine samples will be tested for CT/NG at NARI, Pune.
- Urine samples may be stored at 2-30°C up to 30 days. If the laboratory is not able to test them within this time frame, the specimens must be stored at -20°C.
- Keep your freezer storage and work space organized, this is essential with high volume work.

C. Specimen testing

Refer to the Laboratory Standard Operating Procedures Manual for the following assays to be performed. Test the specimens accordingly as labeled.

1.1 Specimen Management
1.2 Span Diagnostics Quantitative RPR
1.3 Syphagen TPHA
1.4 BioMerieux Trepanostika TP recombinant
1.5 Gen-Probe APTIMA Combo 2 Assay for CT/NG
1.6 J Mitra HIV EIA
1.7 Genedia HIV 1/2 ELISA 3.0
1.8 Calypte HIV-1 BED Incidence EIA
1.9 Focus Diagnostics Herpes select 2 ELISA IgG
1.10 Murex HBsAg (DBS only)
1.11 Murex HCV (DBS only)
1.12 GUD mPCR

The various types of biological tests and the specimens to be collected from the study participants are as follows:

	Name of the disease/symptom	**Test to be done**	**Specimen to be collected**	**Level where the test will be done**
1	Syphilis	RPR (titration)	Blood	District
2	Syphilis	TPHA	Blood	State
3	HIV (prevalence)	ELISA (twice)	Blood	State (Discordant test to be confirmed at NARI)
4	HIV (incidence)	BED-CEIA	Blood	NARI
5	HSV2 (only on 10% of study subjects)	ELISA	Blood or DBS	State
6	NG/CT	TMA	Urine	NARI,
7	HBV (*IDU)*	Murex	DBS	RMRC
8	HCV (*IDU)*	Abbott/Chiron	DBS	RMRC
9	Syphilis (*IDU)*	Trepanostika	DBS	RMRC
10	GUD (only on those reporting an ulcer)	mPCR for TP, HD and HSV	Genital swab	NARI

NOTE: The Specimen Management and Quality Control SOP contain important information and must be thoroughly reviewed by all sites.

D. Specimen Storage

- Samples must be received at the state level laboratory within 6 days of collection. Upon receipt from the district laboratory, the specimens must be verified against the specimen transfer log. Once all specimens are accounted for, the time frame must be verified and documented on the specimen transfer form. Extended delays in transport to the state laboratory may have negative effects on the assays.
- Samples will be stored at the state level laboratories at -20°C until completion of all assays per patient specimen. After completion of tests, samples will be stored in an organized manner and logged accordingly.
- Upon completion, samples will be sent to NARI (Pune) for two reasons: quality control and long-term storage.
- Transport at this time will also include specimen transfer logs. Refer to the Specimen Transport for details.
- **SERUM SPECIMENS WILL ULTIMATELY RESIDE AT NARI FOR LONG TERM STORAGE AT -70°C.**

E. Specimen Transport

- Due to the location of field sites and laboratory sites, specimen movement is inevitable.
- This will be done in an organized manner with regard to the safety of everyone while expediting transfer under optimum conditions to effect a good turn-around time of useful results.
- The IBBA Project has dedicated transportation for samples between field sites and laboratory sites.
- Specimens collected in the appropriate containers in the field will be logged onto a Transfer Log sheet.
- An appropriate robust transporting vessel will be used for sample movement. This will be securely shut and placed in the vehicle for transporting.
- The drivers/laboratory aides will sign out the samples from the clinic and sign them in at the lab after the laboratory technicians have verified the contents and state of the samples.
- As soon as samples are signed for by the lab, they become the responsibility of the lab.

Upon completion of testing at the state level laboratory, samples will be transported to NARI for storage and QC testing.

Samples will be organized properly into cryostorage boxes with specimen box maps created for each. **Boxes once organized will be sealed with tape to prevent opening during transit.** The serum and urine samples will be separated in the following manner:

Serum samples:

- Serum vial (Syphilis)
- Serum vial (-HIV)
- Serum vial (+HIV) – these require Incidence testing (BED CEIA)
- Serum vials (QC)

Urine samples:

- Urine

There will be no long term storage of these samples.

Genital Ulcer Swabs- swabs must be transported frozen to NARI for m-PCR testing.

Dried Blood Spots- each dried blood spot card will be transported in an individual bag. Each bag will be placed in a larger Ziploc bag containing dessicant pouches.

All serum and urine samples sent to NARI must be shipped in a frozen state using dry ice. Samples will be shipped in cryostorage boxes that will be labeled with the following information (this information will also be noted on the accompanying box map form):

- State lab name (ex. NIE)
- Specimen type (ex. Serum HIV/HSV)
- Designated box number

One copy of the box map form will be retained at the state laboratory. The second copy will accompany the shipment in a sealed Ziploc bag.

F. Data Management

The successful management of client test results requires strict daily oversight by the state level laboratory supervisors.

Results for each test will be maintained on laboratory worksheets,/log forms, as well as computer Excel files. There will be one format that all sites will follow to maintain uniformity. All sites must maintain a bound hard copy as well as computer file for all results.

It will be the responsibility of the supervisor to review all test results after the assay has been performed. Before results can be finalized, the supervisor will review the results log sheet and

any instrument-derived printouts (i.e.- microplate reader results, GenProbe CT/NG Leader results, etc.) for comparison and accuracy.

Each log sheet must be checked that each of the following was recorded:

- Technician ID
- Date
- Assay
- Kit Lot Number
- Kit Expiration Date
- Control performance
- Patient results (positive, negative, equivocal)
- Validity of the test run/ Acceptance of results

If the test run is accepted, the supervisor must initial and date the log sheet under the 'Review' comment line. Any instrument printout and specimen log form should be stapled together and stored in chronological order in a binder.

Once test results are finalized, they must be entered into the computer file. This must be done with the utmost of care to avoid transcriptional errors.

NOTE: After any computer entry, the data must be backed up onto a disk in the event of computer failure.

A uniform Excel file has been created to store all test results performed at the state level laboratories. Data that must be entered includes:

- Test date
- Patient ID
- Optical density readings for ELISA tests
- Calculated cut-off or calibrator values
- Confirmatory test results (if required)
- Final test result determination

State laboratories performing the GenProbe AC2 assay will record the result on the proper worksheet on the Excel file.

The main page of the results worksheet is a compilation of all test results for a given patient. Care must be taken in the transfer of data to avoid mismatch of patient test results.

G. Study Management of District Level Laboratories

For the duration of each test period, it will be the responsibility of the state level laboratories to provide assistance and supplies to the district level laboratories. Assistance includes:

- Technical help with quality control of specimen collection
- Supplies for specimen transport and organization
- Quality control of syphilis testing
- Results of syphilis testing must be provided to the district level laboratory in a timely manner
- Supplies for performance of laboratory duties

RESOLUTION OF PROBLEMS:

IT IS CRITICAL GIVEN THE RAPID COLLECTION OF A LARGE NUMBER OF SAMPLES THAT ANY PROBLEM IN SPECIMEN COLLECTION, LABELING, AND TRANSPORT BE ADDRESSED IMMEDIATELY.

H. Quality Assurance Oversight

Quality Assurance for this project is comprised of training, equipment maintenance, testing, Quality Control, proficiency testing (in (QA) ternal/external), documentation, and site inspection.

There are several layers of Quality Assurance that must be monitored and maintained for this project. This includes:

- QA of the district level laboratory by the state level laboratory
- QA monitored within each state level laboratory
- QA of state level laboratories by NARI
- Proficiency testing of state level laboratories administered by NARI/FHI
- Site monitoring visits of state laboratories by NARI/FHI

Refer to the Good Laboratory Practice presentation for what is required for all laboratories to affect proper quality control.

As noted in the section G above, the state level laboratories must monitor the QA of the district level laboratories. This is accomplished by:

- Monitoring the physical state of samples sent by the district lab
- Site monitoring visits (inspection every 2 weeks)
- Monitoring the GLP of the district lab (actual inspection of RPR test records, daily QC logs, maintenance and temperature logs, etc).

QA to be monitored within the state laboratory follows the standards as denoted by GLP:

- All equipment and instrumentation must be operated and maintained as delineated by the manufacturer and noted in the laboratories SOPs.
- Workspace and lab tools must be kept clean and decontaminated daily.
- Assays must follow protocol, and controls used for each run.
- Internal staff proficiency testing can be implemented by blinded testing of known positive/negative samples for each assay every two weeks.

Overall technical assistance for the project will be provided by NARI. To verify test performance from each state laboratory, a percentage of designated QC samples will be tested at NARI, and the results for that patient will be compared to the original results. Any discrepancies will be settled by NARI.

Supportive supervision and monitoring of state level laboratories will occur every two to three weeks by representatives from NARI or the laboratory consultants.

IBBA

STATE LABORATORY SUBMISSION FORM

SPECIMEN TYPE: **Date:** / /2009

State Name:

	1	2	3	4	5	6	7	8	9	10
A										
B										
C										
D										
E										
F										
G										
H										
I										
F										

Total number of participants whose samples have been sent _____

Lab technician's name and signature: _____

Lab Manager's name and signature: _____

To be filled at the Central lab

Name, designation and signature of _____

the person who received the specimen _____

Date and time of receipt _____

Samples were frozen: Yes/ No

Remarks if any_____

IBBA

LABORATORY RESULT FORM (DISTRICT LABORATORY)

Participant's ID Number: _____ District:_____ Group:_____

Study Site: _____ Signature of LT: _____

ENCIRCLE THE CORRESPONDING NUMBER.

Blood (Tested at District laboratory)s	
Date Received: / /2009 Date Tested: / /2009 Laboratory technologist: Name _____Signature_____ Supervisor: Name _____Signature_____	
a. RPR Result	0. non reactive 1. reactive 2. indeterminate
b. If Reactive: Titer	1. 1:1 2. 1:2 3. 1:4 4. 1:8 5. 1:16 6. 1:32 7. 1:64 8. 1:128 9. ABOVE

Remarks: _____

_____

Date:

Laboratory supervisor: Name _____ Signature _____

(Appendix D1)Stock Status of……..

Min. Stock/Re order Level:…….

S. No.	Received			Checked by	Received or Issued by	Issued to					Balance
	Quantity	Date	Indent Form No.			Name	Signature	Date	Indent Form No.	Quantity	
-											