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Protocol

Sexually Transmitted and Blood-borne Infection Prevalence Assessment in High Risk Populations in India

Mapping, Size Estimation and Integrated Behavioral and Biological Assessment (IBBA) in High HIV Prevalence Settings in India

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Family Health International

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
BMGF	Bill & Melinda Gates Foundation
CBO	Community Based Organization
CRS	Chain Referral Sampling
Ct	*Chlamydia trachomatis*
EC	Endocervical
ECS	Endocervical Swab
ELISA	Enzyme Linked Immunosorbant Assay
FHI	Family Health International
FSW-BB	Female Sex Worker – Brothel Based
FSW-HB	Female Sex Worker Highway-Based
FSW-NBB	Female Sex Worker – Non Brothel Based
GUD	Genital Ulcer Disease
HCV	Hepatitis C Virus
Hd	*Haemophilus ducreyi*
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
HVS	High vaginal swab
IBBA	Integrated Behavioral and Biological Assessment
ICMR	Indian Council of Medical Research
IDU	Injecting Drug User
IEC	Information Education Communication
IgG	Immunoglobulin G
MBG	Male Bridge Group
MSM	Men who have Sex with Men
MSW	Male sex worker
NAAT	Nucleic Acid Amplification Tests
NACO	National AIDS Control Organization
NIE	National Institute of Epidemiology
NIMS	National Institute of Medical Statistics
NIN	National Institute of Nutrition
NARI	National AIDS Research Institute
Ng	*Neisseria Gonnorhoeae*
NGO	Non-Governmental Organization
PCR	Polymerase Chain Reaction
PHSC	Protection of Human Subjects Committee
PSI	Population Services InternationalRDS Respondent Driven Sampling
RMRC	Regional Medical Research Council
RPR	Rapid Plasma Reagin
SACS	State AIDS Control Societies
STD	Sexually Transmitted Diseases
STI	Sexually Transmitted Infections
TLS	Time Location sampling
Tp	*Treponema pallidum*
TPHA	*Treponema pallidum* Hemagglutination Assay

Tv	*Trichomonas vaginalis*
VCT	Voluntary HIV Counseling and Testing (for HIV)

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1.0 Background and rationale

In India, sentinel surveillance is used annually to estimate the prevalence of Human Immunodefiency Virus (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. In 2002, sentinel surveillance was conducted at 384 sites and in 2003, at 455 sites.

Despite the HIV, Sexually Transmitted Infection (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 assessment is to gather data for impact monitoring and evaluation of the Avahan India AIDS Initiative funded by the BMGF in 71 districts of 6 States and five highway sites. The proposed mapping, size estimation and integrated behavioral and biological assessment (IBBA) 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 IBBA will be conducted three times during the five-year project period of Avahan. The baseline assessment will be undertaken in 2005, mid-line in 2007 and end-line in 2009. This protocol aims to cover the baseline, mid-line and end-line assessments.

2.0 Objectives

The overall objective of the IBBA is to collect necessary information for assessing the outcomes and the impact of HIV interventions in Avahan project districts. In addition, conduct of the IBBA will strengthen the capacity of national and state level institutes including The Indian Council of Medical Research (ICMR), the National AIDS Research Initiative (NARI), The National Institute of Epidemiology (NIE), The National Institute of

Nutrition (NIN), the Regional Medical Research Council (RMRC), and the National Institute of Medical Statistics (NIMS).

The specific objectives of the IBBA are to collect the following data in selected districts of the Avahan project states of Andhra Pradesh, Maharashtra, Tamil Nadu, Karnataka, Manipur and Nagaland and along the National Highways:

1. To measure the major outcomes and impacts of the interventions funded by the Bill & Melinda Gates Foundation (BMGF) under the Avahan India AIDS Initiative by collecting behavioral and biological trend data in populations targeted by the interventions.

2. To make available data that will be used for estimating sizes of populations targeted by the project.

3. To make information available to a partner organization under Avahan for modeling the impact of the intervention.

3.0 Methods

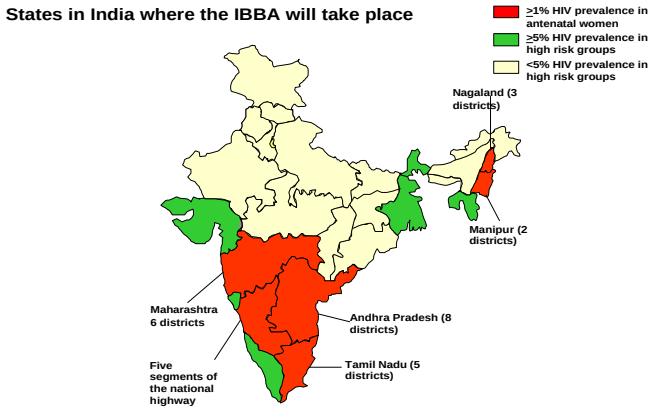
3.1 IBBA populations:
The populations that will be included for mapping, size estimation and IBBA are:

Five Avahan States: Tamil Nadu, Andhra Pradesh, Maharastra, Nagaland, Manipur

- Female Sex Workers Brothel Based (FSW-BB)
- Female Sex Workers Non-Brothel Based (FSW-NBB)
- Male Who Have Sex With Men (MSM)/Male Sex Workers (MSW)
- Male clients of Female Sex Workers
- Male Injecting Drug Users (IDU)

National Highway

- Truckers Drivers and helper (TD/H)
- Female Sex Workers Highway-Based (FSW-HB)



3.1.1 Operational Definitions of IBBA Populations

Female Sex Workers: (Definitions will vary by state/district to match with Avahan program)

a. Female Sex Workers (FSW): Females aged 18 years or older, either brothel or non-brothel based, who sold sex in exchange for cash at least once in the past one month.

b. Female Sex Workers Brothel-Based (FSW-BB): Females aged 18 and years or older working/living/operating in brothels in red light/brothel areas (to be specified for each district) that have been paid cash in exchange for sex at least once in the past month.

c. Female Sex Workers Non-Brothel Based (FSW-NBB): Females aged 18 years or older, soliciting male clients on the street or in other non-brothel settings, who sold sex in exchange for cash at least once in the past one month.

d. Service Bar Based Female Sex Workers (FSW-DSBB): Females aged 18 years or older, soliciting clients in service ("free service") bars, who sold sex in exchange for cash at least once in the past one month.

e. Female Sex Workers Highway-based: Females aged 18 and over who are highway-based, who have been paid cash in exchange for sex at least once in the past month.

Clients of FSWs: Men aged 18 years or older recruited from red-light districts and other commercial sex access points that have paid cash in exchange for sex with a female at least once in the past one month.

Injecting Drug Users: Males aged 18 years or older that have injected drugs for non-medical reasons any time during the past six months.

Men who have sex with men (Definitions will vary by state/district to match Avahan definitions)

a. Male Sex Workers: Males aged 18 years or older who sold sex to other males in exchange for cash (cash/kind in some places) at least once in the past three months. (Hijras/eunuchs to be sampled separately).

b. Men who have sex with men: Males aged 18 years or older who exchanged (bought or sold) sex in exchange for cash (or cash/kind) at least once in the past three months.

Truckers and helpers: Men aged 18 years or older driving trucks or assisting drivers along inter-state transport routes.

3.1.2 Protection of vulnerable subjects

Recruitment of these high-risk populations requires addressing the clandestine, socially marginalized nature of these groups and the behaviors that they engage in. To protect participants who may be vulnerable to coercion or undue influence the following general procedures will be adhered to. These procedures will have added specificity as this protocol is contextualized for each site.

- Access to these groups may require going through various gatekeepers such as employers, brothel owners or police. Discussions with employers will clarify the purpose of the IBBA, e.g. STI detection and treatment, STI counseling, condom distribution and obtaining information to guide improved implementation of ongoing projects. Employers will be made aware that all data forms will have no names, with exception of the consent form which will not be linkable to any of the behavioral or biological data, and that participation by all individuals will be voluntary and through random recruitment. While these employers might be used to gain access, they will not be utilized for any component of recruitment and no information regarding recruitment will be given to these intermediaries. Sites where employers appear to be coercive will be excluded from the IBBA

- Specific efforts will be undertaken to inform the NGOs working with the populations targeted by the IBBA, as well as community leaders, about the purpose, risks and benefits of the IBBA. Prior to recruitment, information about the IBBA will be shared through educational sessions with NGOs and other partners. At these educational sessions the IBBA will be explained and questions answered. These educational sessions will stress the voluntary nature of the IBBA and their importance in tracking progress in reducing HIV transmission.

- IBBA participants will be protected through a voluntary written consent process, with the option of witnessed verbal consent for those who are not comfortable with written consent. All IBBA documents and specimens will be labeled with only a unique respondent number with the exception of the consent form, which will not be linkable to any of the other IBBA documents or data. Prior to recruitment, two IBBA staff will explain the IBBA procedures in detail to potential participants in a private room, and answer all questions. They will emphasize that participation is voluntary and should participants decide not to participate or withdraw from the IBBA at any time, their

decision would not affect any services from the NGO or the clinic that they would normally receive. The IBBA staff will administer written or witnessed verbal informed consent depending on the preference of the participant.

- The IBBA is anonymous. The participant's signature on the consent form is in no way linked to the person's behavioral and biological data since the unique respondent number does not appear on the consent form. No names or personal identifiers will be recorded. All questionnaires and biologic specimens will be labeled with the respondent number. The assessment participant will be given a card with his/her unique respondent number as a way to return for syphilis results and free syphilis treatment, (with the exception of those referred to the Avahan supported Population Services International (PSI)As there are no identifiers there is no way to trace any positive laboratory tests or to determine who chose to participate or not participate in the assessment.

- The IBBA Project staff, India Council of Medical Research (ICMR), FHI and community monitoring boards will closely monitor the consent procedure.

- Discussions will be held between ICMR, National AIDS Research Institute (NARI), FHI, IBBA and Avahan project staff and community leaders on potential impact of data and appropriate release of the data when the IBBA is complete.

3.1.3 Key behavioral and biological indicators

Behavioral Indicators

- Sexual risk behavior including number and type of sex partners ("commercial", "regular" and "non-regular")
- Condom use with different types of sex partners
- Other practices related to condom use and safe sex
- Knowledge of STIs and STI care-seeking behaviors
- Knowledge and attitudes toward HIV/AIDS
- Drug and substance use (including injecting and equipment sharing)
- Mobility and migration patterns influencing sexual behavior and risk
- Perception of HIV and STI risk
- Exposure to Avahan and other HIV/AIDS prevention interventions

Biological Indicators

- STI prevalence (syphilis serology, *N. gonorrhoeae* (Ng), *C. trachomatis* (Ct), herpes simplex virus (HSV) 2 serology, HBC and HCV in IDUs, and for those reporting ulcers *T. pallidum* (Tp), *H. ducreyi* (Hd) and HSV-2 antigen detection)
- HIV prevalence
- HIV incidence (BED-CEIA validated for Indian sub-type C)

3.2 Site and Respondent Selection

3.2.1 Selecting Project Districts for Mapping, Size Estimation and IBBA

Presently Avahan is working in approximately 75 districts across six states of India, 19 districts in Andhra Pradesh, 20 in Karnataka, 16 in Maharashtra, 4 in Manipur, 4 in

Nagaland and 12 in Tamil Nadu. Based on the recommendation of a the WHO M&E Advisory Group for the IBBA, it was decided that the IBBA would be carried out in approximately 29 project districts, 8 in Andhra Pradesh, 5 in Karnataka, 6 in Maharashtra, 2 in Manipur, 3 in Nagaland and 5 in Tamil Nadu. In addition, five segments of the National Highway would also be part of the IBBA.

For selecting the IBBA districts within states, two key criteria were considered: (i) socio-cultural region (SCR); and (ii) size of the female sex worker (FSW) population, or, in the case of Manipur and Nagaland, size of the injecting drug user (IDU) population. Specifically, the requirement was to have representation from each of the different SCRs within a state to ensure heterogeneity in terms of social, economic and cultural characteristics. Then within each SCR the criterion was to select the districts with the highest number of persons at high risk (FSWs and IDUs). A third criterion, prevalence of antenatal clinic (ANC) attendees in the district was originally proposed to fulfill the objective of obtaining a mix of higher and lower prevalence districts. However, in the end, this criterion did not play a role because there were only a handful of cases where more then one district was selected within an SCR, and in those cases, after considering the size of the risk population, there was not much significant difference in terms of ANC prevalence. It is recognized that the size of the risk populations (FSW and IDUs) is fluid and that attempts to enumerate these populations at any given point in time will yield different results. However, since a probability-based method was not being used to select districts, the primary concern was to try to select the districts with the highest concentrations of at-risk populations. The proposed method adequately addresses this need.

3.2.2 Selecting Individual Respondents for the IBBA

Sampling sub-populations of the type of interest for Avahan presents significant challenges. First, lists (or sampling frames) of sub-population members rarely if ever exist. Secondly, because they often represent a small proportion of the general population, obtaining statistically reliable data for such sub-populations through conventional household surveys would require prohibitively large household samples. Third, many of the types of dwellings in which they live (e.g. brothels, migrant hostels, prisons or unconventional dwellings) might not be included in typical household survey sampling frames. Finally, because they engage in behaviors that are illegal or at least stigmatized in many settings, members of such sub-populations are often reluctant to participate in household surveys and risk revealing their behaviors to others who may be present at the time of a survey.

In recent years, a number of sampling approaches have been developed for hard-to-reach and hidden populations that appear to produce survey estimates with acceptable levels of accuracy. Such methods will be used for the IBBA.

The choice of sampling method for individual sub-population members will depend upon the specific group being assessed. Two alternative sampling approaches will be used for the sub-groups included in the IBBA: time-location sampling and respondent driven sampling. (In a few rare situations, conventional cluster sampling will be used for some types of sex workers).

In time-location sampling (TLS), a sampling frame will be obtained through a listing exercise conducted by the IBBA research agency. The listing exercise will use existing information from all existing sources and add to it. From the geographical sites identified during mapping/listing, a time-location sampling frame consisting of venue/time slots will be constructed. Subsequently a random or systematic sample of primary sampling units, (i.e. venue/time slots) will be chosen and data on behavioral and biological indicators will be gathered from a random or systematic sample of population sub-group members appearing at those venues during fixed-length observation periods (e.g., three-hour time segments, entire days or nights). Examples of sites used in TLS for population sub-groups included in the IBBA are brothels, service bars (Mumbai only), street sites where sex workers congregate to solicit clients; and truck stops, border crossings, and bars/restaurants along major highways. Cluster sampling will be used for sex workers and truckers. The specific types of sites to be listed will be finalized after the pre-survey assessment. For MSM/MSW the sampling method will vary by state, and will depend on how the groups are defined in each state, which is related to the way the Avahan program defines and targets them. For clients of sex workers, field trials will help determine which sampling method works best. For IDUs it is proposed to use RDS.

In general RDS will be used for sub-populations for whom significant proportions do not congregate at identifiable sites. RDS is an elaboration of chain-referral sampling (CRS) designed to overcome the major biases associated with CRS methods such as snowball sampling. This is accomplished by, among other things: (1) restricting the number of recruits per recruiter and weighting the data inversely to personal network size to prevent the sample from being dominated by "seeds" and recruits with large personal networks; and (2) providing incentives to reduce non-response bias. We anticipate using this method for sampling IDUs and possibly other groups such as male sex workers or MSM.

3.3 Sample Sizes

3.3.1 Measuring Changes in Behaviors and documenting STI Prevalence

Sample sizes for each population sub-group included in the IBBA have been calculated on the basis of the following factors typically used in surveys with probability samples:

1. the expected baseline value of key behavioral indicators (e.g. consistent condom use with various partner types)
2. magnitude of change it is desired to be able to detect;
3. confidence level;
4. statistical power; and
5. design effect.

The following formula was used to determine the sample size for target groups for the IBBA:

$$n = D\frac{\left[\sqrt{2P(1-P)}Z_{1-a} + \sqrt{P_1(1-P_1)+P_2(1-P_2)}Z_{1-b}\right]^2}{\Delta^2}$$

Where:

D = design effect;

P1 = the estimated proportion at the time of the first survey;

P2 = the proportion at some future date, such that the quantity (P2 - P1) is the size of the magnitude of change it is desired to be able to detect;

P = (P1 + P2) / 2;

? 2 = (P2 – P1)2

Z1-α = the z-score corresponding to the probability with which it is desired to be able to conclude that an observed change of size (P2 - P1) would not have occurred by chance;

Z1-β = the z-score corresponding to the degree of confidence with which it is desired to be certain of detecting a change of size (P2 - P1) if one actually occurred.

For the IBBA, the following assumptions have been made regarding these parameters:

1. Expected baseline value: 50%. Measurements require the highest sample size to detect change when the baseline is 50%, hence this figure was used. If it can be safely assumed that baseline values of all indicators are significantly lower or higher, then sample sizes could be lowered.
2. Desired change to detect: 10-15%. This refers to the amount of change that can be detected between two survey rounds. For example, if condom use changed by an absolute 10-15%, this would be detected as a statistically significant change. A lower absolute change would not be detected as statistically significant. Smaller differences require larger sample sizes.
3. The alpha level has been set at 0.05, corresponding to 95% confidence in the observed estimates.
4. The beta level has been set at 0.10, corresponding to 90% power.
5. Design effect: 1.7 for time-location sampling and 1.5 for RDS. This adjusts for the use of sampling designs that are not simple random methods, e.g. cluster sampling.

Minimum sample size requirements per sub-population per district to measure both differences between groups and changes over time at the levels of significance and power indicated above are summarized in the table below. These numbers will be adjusted upward after the pilot test to account for anticipated non-response (refusal and duplication).

Justification for IBBA sample sizes for key sub-populations

IBBA Population	Indicator	Expected baseline value	Change to Detect	% in denominator	Design Effect	Required sample size	Sample size (rounded off) required for the IBBA
1. FSW Brothel based	Consistent condom use with clients	50%	15%	ALL	1.7 TLS	385	400
2. FSW Non-brothel based	Consistent condom use with clients	50%	15%	ALL	1.7 TLS	385	400
3. FSW Highway based	Consistent condom use with clients	50%	15%	ALL	1.7 TLS	385	400
4. Male Sex Worker/MSM	Consistent condom use with clients	50%	15%	ALL	1.5 RDS	339	400
5. Injecting Drug User	Consistent use of safe injecting equipment	50%	15%	ALL	1.5 RDS	339	400
6 Clients of FSW (Male bridge group)	Consistent condom use with FSW	50%	15%	ALL	1.7 TLS	385	400
7. Truckers	Consistent condom use with FSW	50%	15%	65%	1.7 TLS	592	600

3.3.2 Measuring Changes in HIV Incidence

Based on the BED Incidence EIA testing procedure, individuals tested are classified into three groups:

1. those who are HIV seronegative (N_{neg});
2. those who were infected within the last W days (i.e., 153 days) preceding the test (N_{inc}); and
3. those who were infected more than W days preceding the test ($N_{prevalent\ at\ day\ -W}$ = N_{tested} - N_{neg} – N_{inc}).

For the analysis of incidence, one ignores those in category 3 above, since as they sero-converted longer in the past than the "window" period for the BED-CEIA, they are "non-informative" for the purpose of measuring HIV incidence. Thus, the number of 'informative' participants for the incidence analysis is N_{neg} plus N_{inc}.

The recommended approach for estimating the "incidence (density) rate" ($IDR_{per\ 100\ person\text{-}years}$) (i.e., events per 100 years of risk) and a 95% confidence interval for the incidence rate treats the number of recent infections as a Poisson random variable with total time at risk equal to the time at risk among the sero-negatives (N_{neg} * 153 days), plus average time at risk among the incident cases (N_{inc} * 153 days /2). An alternative measure for summarizing incidence is the "cumulative incidence" (P_t) which is the probability of becoming infected by the end of some time period t (e.g., t=1 year or t=153 days). When the risk of experiencing the event of interest during the exposure period of interest is constant (as is assumed when the Poisson model is applied as above) there is a convenient 1 to 1 relationship between P and IDR as shown below:

$P_{t\ years} = 1\text{-}\exp(-IDR_{t\ years})$ or $IDR_{t\ years} = -\ln(1- P_{t\ years})$

For example, if the $IDR_{1\ year} = .05$ events per person-year (or 5 events per 100 person-years) then the probability that a person who is followed for one year experiences the event within that year ($P_{1\ year}$) is .0488. Alternatively, if $IDR_{1\ year} = .05$ this implies that $IDR_{153\ days} = .02096$ and $P_{153\ days} = .02074$.

Using the BED-IEIA test results, we can estimate $P_{153\ days}$ directly for a cross-sectional sample using the observed proportion $N_{inc} / (N_{neg} + N_{inc})$**.** Thus, if our goal is to test whether two independent groups have the same risk of HIV incidence we can simply compare the observed proportions of participants with an infection within the past 153 days (among the informative participants) for the two groups using standard approaches for dichotomous variables (e.g., a chi-square test for a two by two table). Note that the two groups being compared can be samples from two separate populations, or two independent samples from a given population taken at two different points in time. The latter scenario would apply in the case of measuring changes in incidence over time for the sub-populations of interest for Avahan.

A sizeable number of calculations were run with different combinations of parameters, including underlying annual infection probabilities of between 1% and 25%. These

calculations reveal that on the order of 1,200 to 1,500 subjects per sub-population per round of IBBA would be needed to measure declines in incidence of five (5) percentage points. Thus, measuring changes in incidence for individual sub-populations within IBBA domains (i.e., districts) is not feasible, but is feasible for most groups when aggregate estimates at the state or overall Avahan project level are desired.

3.4 IBBA teams

There will be state specific IBBA teams for each of the six states and one team for the National Highway sites. Each team will have the following constitution.

3.4.1. Pre-survey assessment

FHI will hire three consultants who will:

- Gather and review all the available mapping information, for the selected districts and national highway segments, available from various sources including Avahan partners, NACO, SACS, NGOs/CBOs and different donor agencies. Based on a thorough and critical review of methodologies, coverage and quality of the existing mapping information, the gaps in developing a comprehensive sampling frame for different respondent groups will be identified and filled through a rapid listing exercise coordinated by ICMR and its partners.

- In addition, the consultants will gather information to guide methodological decisions related to the fieldwork for the main assessment.

3.4.2 IBBA:

3.4.2.1 Core team

ICMR will be charged with implementation of the project, with technical support and assistance from FHI. The Principal Investigator will assemble a core team who will direct the IBBA. This team will consist of one principal investigator with senior level medical epidemiological skills and experience, one research coordinator and one research associate with strong knowledge of quantitative research, one senior microbiologist who will supervise the specimen collection and transport procedures as well as the laboratory procedures of the IBBA, and one data analyst who will oversee the data management process in the various states. The number and cadre of the logistic and financial support team will be decided at a later date.

3.4.2.2 State and Highway Teams

There will be six state-wide teams and one highway team. These teams will have overall responsibility for the activities within the state or highway. They will be headed by a program manger with a core team consisting of a field coordinator and a laboratory manager. They will report to the core team regularly and on request. The activities will follow the protocol of the overall IBBA, and the results will be capable of both "standing alone" and being aggregated into the overall program data.

3.4.2.3 District and Highway Sector Teams

There will be multiple field teams in each state and on the highway. The teams will be overseen by district level field coordinators. The composition and responsibilities of the team members are described below.

Supervisor: There will be one supervisor for each field team. The supervisor will be responsible for the overall management of the field team. The responsibilities include ensuring IBBA procedures are properly followed including sampling (with appropriate documentation), accurately completed questionnaires, consent forms signed by the participant or the person obtaining consent and properly witnessed, forms being stored in a secure storage space, and data entry being accurately done. The supervisor will also ensure that the collection and transport of biologic specimens is being conducted correctly and that appropriate syndromic treatment for STIs, consistent with that provided by the Avahan program is made available to the participants. The supervisor will have experience in quantitative research.

Community liaison staff will be attached to each sub-population data collection site. These staff will be recruited locally, and use their local knowledge to assist the IBBA team to select the sample according to the methodology laid out in the protocol. They will also be able to provide information on the studies and reassurance to potential respondents that the principles of confidentiality and anonymity will be strictly adhered to. It is likely that these liaison staff will have been previously working with the community, but current employees of an NGO being evaluated by the IBBA will be excluded. The community liaison staff will have no access to the data and will not handle completed questionnaires.

Interviewers: There will be three to four interviewers on each field team. For populations where time-location sampling is used, a sampler/counter will be responsible for recruiting eligible respondents into the IBBA according to the sampling plan, assigning each respondent to an interviewer, counting eligible respondents throughout the pre-determined time interval, maintaining the sampling flow with random recruitment of respondents and completing the required documentation for the cluster. He/she will need to remain at the site throughout the entire time interval to count respondents, even if the data collection is complete before the end of the time period.

The interviewers will be responsible for ensuring that respondents understand the IBBA procedures, risks and benefits, obtaining informed consent for both the behavioral and biologic components and administering the questionnaire. The interviewer will sign on the informed consent form if the participant gives consent to participate in the IBBA and an additional member of the staff will witness the consent process and sign the form to confirm that the process has been followed in full. The interviewers will be higher secondary literate, with prior participation in large-scale quantitative surveys. All interviewers will receive training in interviewing techniques including how to protect confidentiality and rights of participants.

Medical staff: There will be one medical professional on each IBBA team who will provide syndromic treatment in a manner consistent with the Avahan program, and who

will take ulcer swabs on those respondents reporting symptoms of genital ulcers, if such ulcers are visible upon external exam. A trained medical person will be made available at a nearby referral site where participants will be able to retrieve their syphilis test results approximately a week to ten days after data collection.

VCT: *Results of the HIV test from the IBBA will not be made available to respondents or anyone else at any point of time,* (apart from those anonymously analyzing the data from the IBBA). Respondents wishing to know their HIV status will be accompanied by an individual specifically trained to accompany IBBA respondents who so desire, to pre-selected VCT sites where they can undergo counseling and testing for HIV (using a separate test and separate blood-draw from the IBBA).

Laboratory technician: There will be one laboratory technician on each IBBA team who will be responsible for drawing blood and collecting urine samples from the participant. The technician will ensure that the specimen tubes/bottles are labeled accurately, stored in a cold box, and transported safely to the testing laboratory.

Data entry operators: Due to the large volume of data that will be collected each day as a part of the IBBA, it will be necessary to enter data on a daily basis from the behavioral and biological IBBA procedures. Therefore, a data entry operator will be attached to every district to enable daily data entry that can be consolidated from all field locations into a centralized database.

Once the core research teams and the field teams are identified, there will be extensive training at all levels starting with a project orientation meeting for the core team of investigators, followed by training for field teams (including separate trainings for supervisors, interviewers, phlebotomists, team doctors, community liaison staff, VCT staff, district level data entry staff, district level lab staff and state level lab staff. Core team members will also receive ethics training based on FHI's research ethics training curriculum.

3.4.3 Field Monitoring

There will be several layers of monitoring to ensure that the IBBA is being conducted correctly and informed consent is being obtained according to the approved protocol.

FHI, NARI and the ICMR State Institutes will have ultimate responsibility for ensuring that the protocol is being followed accurately and that ethical standards are maintained. In this aspect the core staff at the district level will be accountable to the senior staff at the state level, and the senior staff at the state level will be accountable to NARI and FHI. The program managers and state level field coordinators will be responsible for close monitoring of the different components of the IBBA. They will make frequent site visits during the fieldwork, ensure adherence to IBBA protocols, and maintain ethical standards. The district field coordinators and supervisors of the field teams will form the last layer of monitoring. They will be responsible for day-to-day monitoring of the IBBA activities, including proper adherence to sampling procedures, completion of consent forms and questionnaires, specimen collection and handling, medical treatment and data entry. They will be expected to take appropriate actions in the case of any breeches.

ICMR, NARI and FHI staff will make at least three field visits to each district in each round of the IBBA

A community monitoring board will be established in each district (or smaller areas as necessary) to liaise between the IBBA sub-populations and the researchers. This group will be composed of individuals accepted and esteemed by their peers (i.e. the sub-population being assessed). Their function will be to monitor and provide feedback to the IBBA staff if procedures are not being handled appropriately.

3.4.4 Follow-up of participants

Follow-up of IBBA participants will be done through the unique respondent number. Following the interview, and collection of biological specimens, the participants will be given a specified date after which they will be able to collect syphilis test results. They will be encouraged to go to specified referral clinics to collect their syphilis results. The benefits of returning for these results include free treatment in case of a positive syphilis test and further counseling and education for a safer and healthier life.. Results of syphilis tests and treatment for those who are infected will be dispensed at participating Avahan clinics where possible, and other clinics where Avahan clinics are not in close proximity to the IBBA sites. Respondents who wish to be tested for HIV will be accompanied by NGO staff or other persons identified and trained for this purpose (if they so desire to pre-identified VCT sites where they can receive pre-test counseling, *repeat* HIV testing (results from the IBBA HIV test will not be made available for this purpose) and post-test counseling and referrals for further HIV/STI prevention, care and support services. The NGO (or other) staff will facilitate access to the VCT sites and serve as advocates for the participants. Post-test counseling for HIV will be done for every participant who goes to the VCT site, irrespective of the test result.

Training on STI counseling will be provided to participating NGO (and other) clinic staff. The VCT services to be used during the IBBA will be identified and strengthened as part of the IBBA preparatory work and will be monitored throughout the assessment.

4.0 IBBA procedures

4.1 Sampling Frame Development

A list of Primary Sampling Units (PSUs) will be needed for those sub-populations for which time-location cluster sampling is used. This is anticipated for all sub-populations with the exception of IDUs and MSM. The sampling frame will consist of physical locations where sub-population members congregate. The sampling frame must be complete, meaning that all such locations must be included and the list of locations should be up-to-date and reflect the current configuration of venues where the sub-population can be accessed. An incomplete or out-of-date sampling frame would increase the risk of obtaining a biased sample and should therefore be avoided. In addition, the types of sites to be included in the sampling frame will need to be specified in advance. For example, if the pre-survey assessment suggests that female sex workers can be found in brothels, bars, discos, hotels, lodges, parks, street corners, hair salons, bus stations and train stations, a decision will have to made whether to include ALL such

types of sites in the sampling frame, or only a subset. This delineation will be documented and maintained over subsequent rounds of the IBBA to ensure consistency over time.

In some instance it will be decided to have multiple categories of sex worker, for either stratified sampling or separate sampling domains. It is recognized that sex workers often "cross-over" different types of sex work (e.g. brothel-based, street-based, residence based, etc). Information about this will be obtained in the questionnaire so it can be accounted for in the analysis.

In addition to the list of sites, information concerning important characteristics of each site will be needed to guide the sampling design. These characteristics include: times of day when sub-population members can be accessed at that site; any distinctive differences in the sub-population members frequenting the site at different times of the day or days of the week; information regarding whether the same sub-population members tend to frequent the site everyday or whether there is turnover; information regarding whether sub-populations frequenting this site also frequent other sties; and an approximation of the number of sub-population members that can be expected to be present at the site at different times of the day, week or month. Any seasonal patterns will be noted as well.

Developing such a list will require participation and collaboration by individuals and/or organizations that are close to the sub-population or part of the sub-population. This may include organizations that are conducting programs or providing services for the population. In fact, such a list or partial list may already be available and usable as a starting point for a sampling frame. But disclosure of such lists can be sensitive, just as facilitating access to the community can be sensitive, which is why it is so important to build a trusting relationship with the community ahead of time.

Gathering information to construct the sampling frame will be one of the first encounters between the research team and the sub-population members. In parallel to this step a series of community preparation activities will take place to share information with key partners and gatekeepers about the purpose of the IBBA. The activities will include discussions about what the IBBA will entail, its risks and benefits to the community and measures to protect the privacy of those who participate in the IBBA. The researchers will be prepared to respond to inquiries about the details of the IBBA and to receive input on how to ensure its success.

4.1.1 Size Estimation

Size estimates will be done in the 29 districts selected for the IBBA plus the national highways. The groups for which size estimates will be done are:

1. Female Sex Workers Brothel-Based - (FSW-BB);
2. Female Sex Workers Non-Brothel- (FSW-NBB);
3. Male sex workers (MSW) or men who have sex with men (MSM);

4. Injecting drug users (IDU);
5. Truck drivers and helpers (TD/H); and
6. Female Sex Workers Highway-Based (FSW-HB)

(Note: Size estimates for clients of sex workers will have to come from household surveys conducted outside of the IBBA, e.g. National Behavioral Surveillance Survey by NACO and National Family Health Survey by Ministry of Health and Family Welfare).

A combination of methods will be used for size estimates. It is generally advisable to use multiple methods because of the limitations of any one method. The choice of methods will depend on the population and the local situation and will be guided by the findings of the pre-survey assessment.

1. **Enumeration** – In populations where a large proportion of the "members" can be found at sites or venues that are relatively stable i.e.: 1) not too much movement of sub-population members between sites; and 2) sub-population members are present at the sites most of the time, then an enumeration (headcount or census) can be done. Such a count would involve visiting all sites/venues where sub-population members congregate for several days in a row and counting the number of sub-population members present. This information can be supplemented by key-informants. If this method is selected the enumeration can be done during sampling frame development for the IBBA.
2. **Multiplier** – using IBBA data (whether by RDS or TLS) combined with service delivery or program statistics. The ability to use this method will depend on the quality of the data obtainable from local NGOs or service providers. The method will be possible only if NGOs (e.g. Avahan projects or other service providers) collect data on the number of *individual clients* participating in their programs/interventions over specified periods of time and if the clients are identifiable by type (e.g. FSW-BB, FSW-NBB, MSW, etc), and if the data are recorded/classified by type of client. If data is collected on visits or number of contacts as opposed to individuals then the data will not be usable for size estimation. These issues are being investigated in detail during the pre-survey assessment. If the multiplier method is used then questions in the IBBA will have to be carefully constructed to capture whether respondents access the services being used to obtain the multiplier.
   Note: An alternate method of obtaining the multiplier might be possible if high quality service statistics are not available. This alternate method would involve "tagging" potential respondents ahead of time (by giving them a "gift" that they will remember…such as a keychain or an armband), and then documenting the proportion who report having received the gift as part of the IBBA. This would then serve as a multiplier.
3. **Direct survey estimate with RDS adjustment** – This method would entail direct size estimation based on IBBA data collected using venue-based sampling. The limitation would be that it would not capture sub-population members who do not frequent the types of venues included in the IBBA sampling frame. To address this limitation, a separate abbreviated RDS assessment could be conducted for the

purpose of finding out what proportion of sub-population members do not access the types of venues included in the IBBA sampling frame. This would provide a multiplier that would allow for an adjustment to the direct size estimate. This method would require that two IBBAs be done, the TLS sample for the behavioral and biological data and the RDS for the multiplier. Reduced sample sizes for the RDS assessment might be possible. Given the excess time and resources required, this approach might be used in a limited number of sites on a trial basis to see whether it is useful enough to consider for size estimation in other sites.

4. **Capture-Recapture** – This method involves "tagging" members of a sub-population at given locations during specific periods of time and then repeating the exercise at a later period in time. Based on the overlap (people who are tagged and retagged) and those not retagged, the size of the population can be estimated. The method relies on a number of assumptions including: 1) samples taken at time one and time two must be independent of one another (i.e. not correlated); 2) each member of the population should have an equal, non-zero probability of being "captured"; 3) the individuals identified in both captures must be correctly identified as recaptures, and no-one else should be identified as a recapture; and 4) there should be no major in-migration or out-migration from the population between the initial and the second capture. Given the difficulties of not violating these assumptions, this method is likely to be of limited use. However, it may also be trialed in a limited number of sites.

Based on the information currently available, the table below indicates the proposed method of size estimation for each population, subject to change after the pre-survey information is available.

Population	Sampling method for IBBA	Proposed size estimation method	Alternate method (If good service statistics not available)
FSW-BB	TLS	Enumeration plus multiplier using IBBA data and service statistics	Direct estimation
FSW- NBB	TLS	Multiplier using IBBA data and service statistics	Direct estimation
MSW/MSM	RDS	Multiplier using IBBA data and service statistics	Direct estimation coupled with RDS
IDU	RDS	Multiplier using IBBA data (from RDS sample) and service statistics	
Truck drivers and helpers	TLS	Multiplier using IBBA data and service statistics or truck company data	

FSW-HB	TLS	Enumeration plus multiplier using IBBA data and service statistics	

4.2 IBBA Procedures

4.2.1 The behavioral assessment

For the behavioral assessment, standardized behavioral questionnaires for the different sub-groups included in the IBBA will be used. Care will be taken to ensure that the data needed for impact modeling are also gathered in the IBBA. The information that will be obtained using the behavioral questionnaire will include:

- Socio-demographic characteristics;
- Sexual history and practices (penile-vaginal, oral, anal);
- Types of sex partners;
- Condom use with different type of sex partners;
- Knowledge of STIs and STI care-seeking behaviors;
- Knowledge and attitudes toward HIV/AIDS;
- Sex purchasing and selling behaviors among males with other males;
- Needle, syringe and drug sharing practices among IDU (and other groups who may inject such as FSWs);
- Questions related to mobility; and
- Exposure to Avahan and other HIV/AIDS prevention interventions

The questionnaires will be adapted to local needs (language, terminology) and will be pre-tested in all IBBA areas. Before pre-testing, the questionnaires will be checked for appropriate sequencing and skip patterns and translated to the local language(s) of each state, and back translated to check the accuracy of the translation. Pre-testing the questionnaires will include checking to make sure that the questions are interpretable in a commonly understood manner and that the original meaning of the questions have been kept intact in the process of adapting them. An instruction manual will be developed for interviewers and supervisors which will go through the questionnaire, one question at a time, explaining in full, the rationale behind each question and its intended meaning

4.2.2 Clinical Procedures

IBBA procedures for STI testing and treatment and HIV testing and counseling are outlined in the table below.

Venous blood draw on FSW, MSW, MSM, Clients of FSW • HIV (prevalence/incidence) • Syphilis (RPR≥ 1:8, TPHA) • HSV2 on subset Dry blood spot IDUs • HIV (prevalence/incidence), HBV, HCV, syphilis (Treponostika)

First catch 20 ml urine - prepare and store for Ng/Ct testing using nucleic acid amplification tests (NAATs) Self administered vaginal swab – prepare and store for Ng/Ct using NAAT
Ulcer swab • mPCR for TP/HD/HSV2 if report external clinical ulcer
Treatment and follow-up • Syndromic management for STIs consistent with Avahan guidelines • Refer to Avahan clinic if report symptom of ulcer which cannot be identified by external examination • STI and HIV Education • Provision of syphilis serology test results and free treatment if positive • Escort to VCT centre for retesting (for those who so desire)

The decision to obtain urine for a self-administered vaginal swab will depend on the final decision regarding which NAATs test kit is chosen for the study sites and the willingness of the participants to take a self administered swab. There is extensive experience globally with self-administered vaginal swabs as a well accepted, easy method to obtain specimens for NAAT testing

4.2.2.1 Rationale for STI/HIV testing

As part of the IBBA participants will be tested for STIs and HIV. Following consent procedures (see details below), a venous blood sample and a urine specimen or self-administered vaginal swab will be collected. For IDUs a dry blood spot will be obtained in the place of venous blood. All testing will be linked anonymous testing whereby all specimens are linked only to a unique respondent number.

Participants will be given this number and told that they need to keep the number in order to obtain their syphilis test results, if they so desire. This number system will be supplemented with a personal password/codeword system in the case that the number is lost.

After completion of the behavioral questionnaire and collection of specimens, sex workers (both male and female) will be provided with syndromic STI treatment in a manner consistent with the Avahan program. Ulcers swabs will be taken from all who both report ulcer symptoms and are found upon external examination to have a genital ulcer. Treatment will be given and ulcer swabs transported with the urine and blood samples. More complex cases (e.g. internal ulcers or other symptoms), if they agree, will be accompanied to NGO clinics for more comprehensive evaluation and treatment. Results of syphilis serologic testing will be available at the NGO clinics at a specified time after recruitment. Since results will be labeled with the identification number only; participants will need to present with their numbered card to obtain syphilis results. NGO outreach and peer educators will enforce the importance of returning for syphilis results and treatment in the case of a positive result. All participants will know through the consent process that their blood is being drawn for syphilis and HIV testing and that it will be stored for potential future research on HIV infection.

IBBA respondents wishing to know their HIV status will be facilitated by an individual specifically trained to accompany IBBA respondents who so desire, to pre-selected VCT sites where they can undergo counseling and testing for HIV (using a separate test and separate blood-draw from the IBBA). This facilitation will include accompanying the participant to the VCT site and paying transport costs and user fees if necessary. The NGO outreach worker/peer educator will act as an advocate for the participant at the VCT site as well as reporting back on the quality of the service with respect to accessibility.

The VCT services to be used during the IBBA will be identified, strengthened and monitored as part of the IBBA preparatory work. During the pre-survey assessment for the IBBA sites, existing VCT services – both government and private, will be identified. In sites where there are no readily accessible VCT sites, a temporary VCT site will be set up. Based on key informant interviews with health providers and others in the community, the ones providing the best quality of services will be identified from multiple sites. These VCT services will be approached and asked to participate as referral counseling sites. Those that agree to participate will undergo refresher training in HIV counseling as well as sensitivity training for issues of the target populations (including IDU, MSW and FSW). Community representatives will be part of this training. As part of site preparation, clear referral networks will be documented for HIV infected persons for referral from the VCT site and NGOs will also document service referrals at their sites if they are at significant distance from the VCT site.

Strengthening the existing VCT sites to provide results to IBBA participants increases costs and management complexity for the IBBA but has several advantages:

- It uses and strengthens existing infrastructure that is likely to be sustainable.
- It will make subsequent rounds of IBBA easier to implement.
- It will build relationships with government sectors.
- It will enhance the services of the government sites to make them more accessible to marginalized populations.

In addition, use of independent VCT sites addresses the expressed preference of members of the community not to have the NGOS working with them to know their HIV status

The monitoring will be done by the NGO staff accompanying the participants (for those participants who want to be accompanied) and periodic assessment visits by ICMR and FHI.

Details of specimen collection and clinical procedures

The clinical procedures involved in STI prevalence studies are standard medical procedures for clinical examination and clinical specimen collection. All personnel involved in clinical procedures will be medical personnel.

- Collection of 20 ml of first-catch urine, at least two hours since last void, or self-administered vaginal swab for nucleic acid amplification testing for N. gonorrhoeae and C. trachomatis.
    - Collection of up to ten milliliters (generally 7ml in a vacutainer) of venous blood to test for HIV and syphilis serology. A subset will have stored serum tested for HIV incidence (BED-CEIA), and HSV2. Remaining sera will be stored in the short term for quality control testing and for those participants who consented for long term storage.
-
- For patients reporting GUD symptoms, an external examination will be performed including inspection of genital and anal areas and local lymph nodes looking for local changes: erythema, warts, abrasions, ulcers, swelling and discharge. All genital and anal ulcers identified will be sampled with a Dacron swab for *T pallidum*, HSV-2 and *H ducreyi* using multiplex PCR.

At the conclusion of the IBBA activities, the participants will receive group education on STIs and HIV, be provided with IEC material and information about NGOs/CBOs in the district, condom outlet points and other service providers such as health care providers, and legal aid.

4.2.2.2 Consent procedures

A detailed and standardized consent process will be conducted for each respondent. Respondents will be asked for written consent to participate in the IBBA with an option of oral witnessed consent if they prefer. The purpose of the IBBA and the IBBA procedures will be explained in simple and understandable terms, in the local language. The potential participants will be informed that all information and discussions will remain confidential, their participation is voluntary, they may refuse to answer any questions and that they may leave the IBBA at any time. They will also be informed that their non-participation will not affect medical treatment if they seek it. The prospective participant will be asked to explain the activities of the IBBA back to the interviewer and witness to confirm an understanding of the procedure. The potential respondent will be asked to give written consent for the behavioral questionnaire and the biologic samples separately. If the prospective participant does not wish to give written consent, he/she may also give oral witnessed consent to participate in the IBBA In the case of oral consent, once the members receiving the oral consent is convinced that the participant understands what has been agreed upon, the interviewer and the witness will sign on the consent form that declares:

> *"I have read and explained this informed consent form to the IBBA participant in (name of local language). The participant has explained the IBBA activities back to me and I am convinced that the participant understands the activities that will occur. The participant has not been coerced to participate and has given oral consent to participate in all aspects of this IBBA".*

If the participant refuses to participate, the IBBA staff will respect the respondent's rights and thank them for their time.

4.2.3 Field data flow

Forms for each respondent will be kept in a folder. Each respondent will be given a unique respondent number. All forms for a given respondent will contain that number with the exception of the consent form. The questionnaire will contain information on location, time and date of interview, the name of the consent staff member, interviewer, doctor, supervisor and data entry clerk(s). The folder will contain:

- The consent form (signed and witnessed); and
- The completed questionnaire.

The supervisor will sign the cover of the folder after checking forms for accuracy and completeness. Immediately after checking the forms the consent form and the completed questionnaire will be separated. The folders will be taken to a secure location each night.

4.2.4 Field collection, Transport and Storage of Biological Specimens

Approximately 10 milliliters of venous blood will be collected in red top vacutainer tubes and labeled with the participant's unique number and date of collection. This will be placed in a cold box that will be transported to the district laboratory, at the end of each IBBA session each day. At the district laboratory, the specimen will be centrifuged, the serum aliquoted into four tubes and labeled with the IBBA respondent number and date of collection. After performing the syphilis tests, the serum will be retained for confirmation and quality control. All samples will be frozen for transport to the State Laboratory.

First catch urine samples (20ml) will be transported to the District Laboratory where they will be aliquotted into three 1ml tubes, and then frozen for transportation to the State Laboratory.

4.3 Laboratory Procedures

Blood, swabs and urine specimens will be picked up by the laboratory technicians for laboratory tests.

- HIV: Screening for HIV will be done using enzyme-linked immunosorbent assay (ELISA). A positive HIV screening test will be confirmed with a second ELISA and then undergo the BED-CEIA test (see below).
- Syphilis serologic testing performed on sera using a quantitative rapid plasma regain (RPR) screening test with a qualitative Treponema Pallidum Hemagglutination Assay (TPHA) confirmation test.
- Polymerase chain reaction (PCR) for *N. gonorrhoeae, C. trachomatis.* First catch of 20 ml urine will be stored in a cool box until they are transported for processing to

laboratories with capabilities to perform PCR tests.

- Multiplex Swab on reported and clinically identified genital or anal ulcers. Dacron swab stored dry at -70^{o}c. Subject batched specimens to multiplex testing for *T pallidum*, *H ducreyi* and Herpes simplex Type 2.
- HSV-2 serology.
- HIV incidence testing: The newly developed BED-CEIA (HIV-1 subtypes B, E, and D, IgG-Capture Enzyme ImmunoAssay) that has also been validated with the Indian sub-type C assay will be used for measuring incidence of HIV.

4.3.1 Laboratory Sample Allocation and Management

At the district laboratory, the specimen will be centrifuged and divided into four aliquots of serum. One of the aliquots of serum will be used for syphilis testing locally, and the remaining aliquots stored frozen in preparation for transport to the state level laboratory.

The state laboratory will carry out the activities outlined and act as a staging station for frozen samples destined for the central laboratory.

The central laboratory (NARI in Pune) will receive and store all samples from the state laboratories, and carry out HIV incidence testing. All quality assurance activities will be managed from the central laboratory.

Long term storage of specimens: A protocol will be developed by ICMR and NARI on long-term storage of specimens so that specimens could be used for additional testing at a later time.

4.3.2 External Quality Assurance

The standard operating procedures and basic minimum standards will be agreed with NARI, who will draw up an implementing laboratory supervision and quality assurance schedule. Ten per cent (10%) of all samples will be re-tested in a different laboratory according to an agreed QA panel construction.

4.4 Data analysis

The questionnaires for the IBBA will be checked daily for completeness and accuracy by the field team supervisors and transported daily to a central location. At this center, the questionnaires will be stored in a secure place. Data on the questionnaires will be entered into EPI-INFO (or other appropriate data entry program) on a daily basis. Cluster information (needed for analysis) will also be entered into an appropriate database on a daily basis. All biological specimens will also be labeled with the corresponding unique identification number. Results of the laboratory tests will be entered into the database as and when the test results are available. Checks will be built into the data entry software to avoid data entry errors. Double data entry will be done to maximize accuracy. Analysis of

the IBBA data will be done in STATA for the TLS samples and with RDSAT for the RDS samples.

4.5 Pilot test

Subsequent to pre-testing and finalizing questionnaires, a full pilot test will be conducted in one of the IBBA districts of each state. The pilot test is meant to be a "dry-run" of the entire process prior to the main survey. Its purpose is to test the process (focusing on sampling and operational procedures) before going to scale. The pilot will:

- Be done in one IBBA Avahan district in each state and will be staggered so that sex workers (female, male, transgender) are done first. It is anticipated that the first pilot will take place in AP.
- Include 25 respondents per group (in each state).
- Allow for testing the sampling strategies for different groups.
- Allow for testing the specimen collection and transport procedure.
- Allow an estimation of the refusal and duplication rate to guide required sample size inflation for the main IBBA where necessary.
- Allow testing of the consent procedure.
- Include information (lessons learned) that can be incorporated into the field guidelines for the main IBBA

Note: In populations where RDS sampling is used a full pilot of the sampling process will not be possible. However, the rest of the IBBA process can be piloted.

5.0 Potential risks and benefits

5.1 Potential Risks

All efforts will be made to minimize the risks to the participants.

There will be no physical risks involved for any of the participants during the data collection process (pre-survey activities, construction of sampling frame, collection of behavioral data). There are no physical risks that are more than any risk of a routine STI examination and venipuncture. There are minimal risks of bleeding and bruising related to venipuncture. Physical risks involved in collecting blood by venipuncture and while obtaining swabs specimens will be minimized by the use of medically trained personnel to draw blood and collect biological samples. The IBBA will also ensure adequate supply of new, sterile disposable needles. Puncture-proof containers will be used for needle disposal and biological waste will be disposed of in an appropriate manner.

The interviews conducted during the pre-survey activities, and development of the sampling frame, will focus mainly on group and location characteristics. Therefore, there

is no perceivable individual harm done to individuals of the sub-population during this phase of the project. However, the exercise might result in the group or the locations mapped becoming more conspicuous leading to stigmatization or harassment of the group by law enforcement agencies and others. There is a possible psychological risk due to the sensitive nature of the questions in the behavioral and biological questionnaire. The questionnaire will be administered in a private setting, will not contain identifiers and the participants will be told that they can refuse to answer any questions. There are also some psychological risks from learning that one is infected with a STI or HIV. This will be handled by providing counseling about STIs/HIV, and reduction in HIV-related risk behavior.

There may be social risks of being diagnosed with a STI. In general the psychological and social risks of being diagnosed with HIV infection are more severe than with a diagnosis of other STIs. The IBBA protocol will pay particular attention to confidentiality procedures and will ensure high quality training of IBBA staff and HIV counselors. Participants will be assigned a unique respondent number at the time of enrollment. No identifiers will link STI results with IBBA participants.

5.2 Potential Benefits

At an individual level, the participants will be treated syndromically on the day of the IBBA for STIs in accordance with the Avahan STI Clinic Operating Guidelines and Standards The IBBA will contribute to the improvement of the health of the individuals and their health seeking behavior through counseling by the IBBA team doctors. Participants will be referred for retrieval of syphilis test with free treatment as necessary.

By providing enhanced access to HIV voluntary counseling and testing services to those who so desire, the IBBA can contribute to the positive living of people who test positive for HIV and enable HIV secondary prevention efforts. Those who test negative will be counseled to eliminate their risk of HIV infection by adopting safe behaviors. The information obtained from the IBBA will be used in designing HIV prevention interventions for the population sub-group and also provide care and support services for those infected and/or affected by HIV.

XXXXX. Security policy for Protecting Survey Population and Confidentiality

A uniform security and confidentiality policy for protecting study population will be followed throughout different procedures of IBBA. This includes information regarding study design, study population, mapping of study sites, strategies for implementation for the study, information on time and location of sampling, consent forms with personal identifiers, interview including sexual and behavioral information of enrolled survey population and biological specimens. The security and confidentiality will be ensured at all steps by following measures:

1. State ICMR institutes whenever subcontracts a research agency for implementation of any IBBA activity including mapping, sample frame preparation, sampling, interviews and specimen collection will sign an agreement with ICMR institutes (see Annex XX). The research agency will follow the agreement and ICMR institutes will

ensure implementation of the agreement. Any breach in agreement will be reported to ICMR ethical review committee and FHI IRB.

2. The research agency will inform each and every employee regarding the confidentiality policy and each employee will signed oath of confidentiality (see Annex XXX).

3. A data and specimen Security policy for Protecting Survey Population Confidentiality guideline is being developed for ensuring safety and confidentiality al all level. This includes data and specimen safety at field after collection, at district laboratory and district offices of research agencies, at state ICMR institutes and ICMR laboratories. ICMR will ensure the implementation of the guideline at all level (see Annex XXX).

6.0 Ethical review

The protocol and draft questionnaires will be reviewed for approval for the three rounds of the IBBA – baseline in 2005, mid-line in 2007 and end-line in 2009 by the institutional ethics committee of each partner institute that will implement the IBBA in different locations. In addition, the protocol will be submitted to the Health Ministry Screening Committee of the Ministry of Health and Family Welfare and to the Protection of Human Subjects Committee (PHSC) of Family Health International. In addition to the protocol, the consent forms and the draft questionnaires will also be submitted for approval to the PHSC. Approval will be obtained from all the above-mentioned review bodies prior to subject recruitment.

7.0 Monitoring plan

Explicit monitoring plans will be developed to ensure that the IBBA teams adhere to all provisions designed to protect the rights of voluntary participants during the assessment. ICMR and FHI will be responsible for the overall monitoring of the IBBA. This will include:

- Review IBBA procedures to ensure that the protocol guidelines are followed and any amendments if required are approved by the appropriate committee;
- Verify that informed consent was correctly administered for all participants by reviewing signed copies of the consent forms;
- Ensure adverse event reporting and that any participants experiencing adverse events have been appropriately treated and referred for further care if necessary;
- Conduct monitoring visits during the fieldwork;

- To preserve confidentiality of participants ensure that the IBBA data collection instruments and biological samples are being stored properly and that only the necessary staff have access to that data
- Ensure data entry is being done appropriately; and
- Oversee the process of data analysis and report writing.

8.0 Ethical Issues

Keeping in mind the sensitive nature of the IBBA top most priority will be attributed to the protection of participants at all phases of the assessment and during dissemination of the results. The IBBA has been designed to maximally protect the participants balanced with the individual benefit and community benefits from this IBBA. Specifically,

- Participation will be voluntary with subjects free to withdraw at any time. Withdrawal will not affect services they would normally receive.
- Informed consent is written with the option of oral witnessed consent. The consent form will be translated and pilot tested. This consent will be translated into the local language. Participants will be offered a written statement regarding the research. The ethical committee of the ICMR in India felt that written consent is the preferred method to obtain consent for IBBA. They however realized that given the clandestine and potentially illegal nature of some of the behaviors of the study participants that signing consent may deter participation. As such, the committee also wanted the option of a witnessed oral consent. Use of oral consent is appropriate as per 45 CFR 46, 46.117(c)
- No names will be recorded. All documentation is anonymous, linked only by a unique respondent number.
- IBBA staff will be trained in discussing sensitive issues and protecting participants' confidentiality and human rights.
- Specific procedures have been developed to ensure that there is maximal anonymity and that there are no "employer" reprisals for non-participation.

9.0 Dissemination plans

A written report of the results of the IBBA will be provided to the Ministry of Health, the National AIDS Control Organization, SACS, Bill & Melinda Gates Foundation Avahan India AIDS Initiative, Avahan Partners, and other NGOs and agencies in the country working on STI and HIV issues. Attention will be given to the sensitivities attached to the social risk of those groups included in the IBBA when preparing reports. At the end of the IBBA, a one-day workshop will be held in each of the five states to disseminate the findings from the assessments. Workshops will also be held to disseminate the findings from IBBA on the National Highways. An oral debriefing will be done with key interested parties including relevant ministries, other donors and key NGOs, and specific disseminations will be done for the population sub-groups included in the IBBA. Results from this assessment will be presented at national, regional and international meetings and published in international peer-reviewed journals.

Dissemination of HIV prevalence levels from the various populations at the district level will not be made publicly available. Circulation of these district specific data will be limited to Avahan, FHI, ICMR, NARI and selected State Government partners. Only data aggregated at the state level on HIV will be disseminated publicly.

10.0 Project outputs

10.1 IBBA outputs

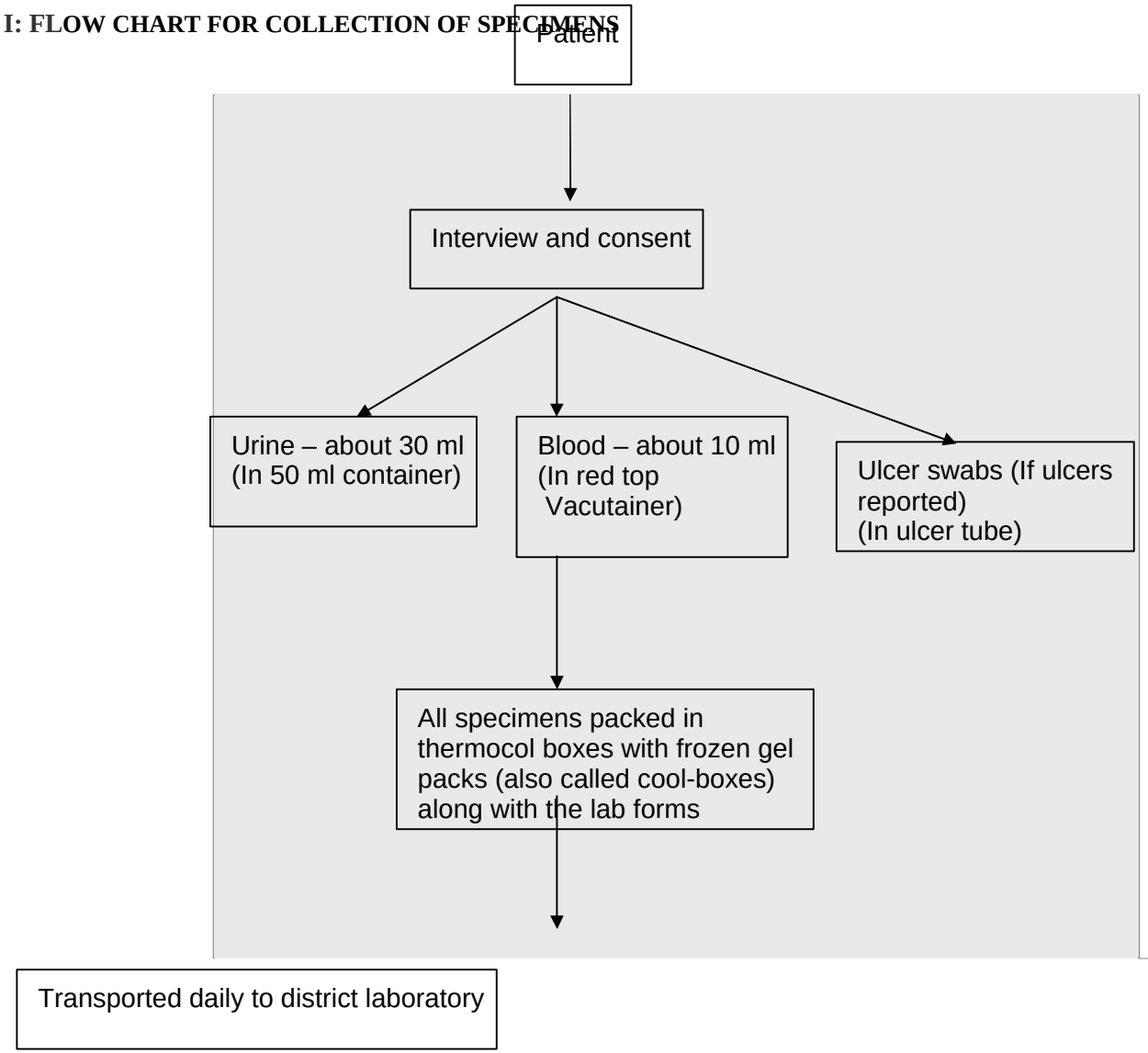
- A measure of size of each sub-population in each districts included in the IBBA will be obtained. This information will feed into the models to gain estimates of number of infections averted.
- Key HIV, STI and drug injecting risk behavior indicators and related knowledge and intervention exposure indicators, as outlined in section 4.2.1.
- HIV prevalence and incidence data.
- STI prevalence data.

This data will be critical to the construction of the models which will yield the overall impact numbers, in terms of infections averted.

	Year 1			Year 2				Year 3				Year 4				Year 5			
Months **Activity**	**1-6**	**7-9**	**10-12**	**13-15**	**16-18**	**19-21**	**22-24**	**25-27**	**28-30**	**31-33**	**34-36**	**37-39**	**40-42**	**43-45**	**46-48**	**49-51**	**52-54**	**55-57**	**58-60**
Start-up																			
IBBA																			
Pre-Survey Assessment																			
Development of the questionnaires																			
Development of sampling frame																			
Laboratory assessment																			
VCT assessment																			
Pre-testing																			
Pilot Assessment																			
Training of the investigators and supervisors																			
Fieldwork																			
Data analysis																			
Report writing																			
Dissemination of the findings																			
Annual Behavioral Assessment																			

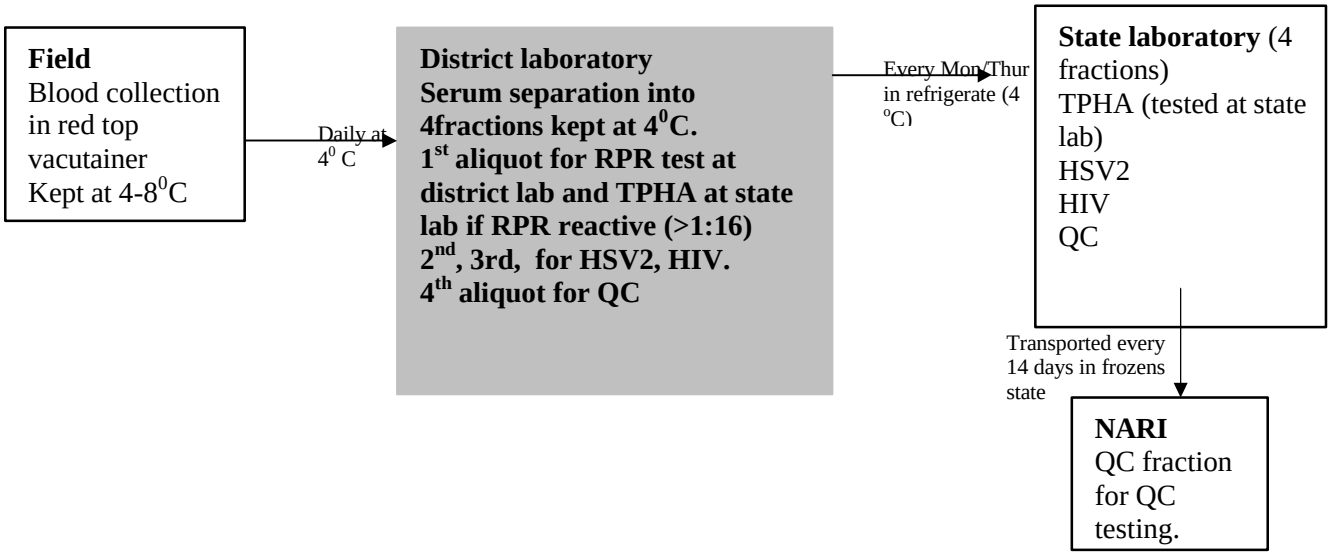
Development of the protocol																			
Development of the questionnaires																			
Pre-testing of the questionnaires																			
Training of the investigators and supervisors																			
Fieldwork																			
Data analysis																			
Report writing																			
Dissemination of the findings																			

APPENDIX I: FLOW CHART FOR COLLECTION OF SPECIMENS



Comment [t1]:

FLOW CHART FOR BLOOD HANDLING



FLOW CHART FOR URINE HANDLING