ABSTRACT: Introduction: This paper details the methods used in the second national Biological and Behavioral Surveillance Survey (BBSS) of HIV, syphilis, and hepatitis B and C among men who have sex with men in Brazil. Methods: Respondent-driven sampling (RDS) was used in 12 cities in 2016. The targeted sample size was initiated with five to six seeds in each city. HIV, syphilis, and Hepatitis B and C rapid tests were offered to participants. RDS Analyst with Gile’s successive sampling (SS) estimator was used to adjust results as recommended and a weight for each individual was generated for further analysis. Data for the 12 cities were merged and analyzed using Stata 14.0 complex survey data tools with each city treated as its own stratum. Results: Duration of data collection varied from 5.9 to 17.6 weeks. 4,176 men were recruited in the 12 cities. Two sites failed to achieve targeted sample size due to a six-month delay in local IRB approval. No city failed to reach convergence in our major outcome variable (HIV). Conclusion: The comprehensive BBSS was completed as planned and on budget. The description of methods here is more detailed than usual, due to new diagnostic tools and requirements of the new STROBE-RDS guidelines. Keywords: HIV Antibodies, Sexual and Gender Minorities, Brazil, Statistics, Methods
INTRODUCTION

Rising HIV seroprevalence rates for men who have sex with men (MSM) continue to be reported globally\(^1\)\(^-\)\(^8\). Evidence can be found in Brazil as well. The incidence rate of AIDS cases among men 15 – 19 and 20 – 24 years of age increased from 3.7 to 6.9 and from 18.1 to 33.1 (/ 100,000) from 2008 to 2015 in the country\(^9\). While the estimated prevalence of HIV among the population of 15 years of age or older is 0.37%\(^10\), recent data suggest HIV rates of 4.9, 5.9, and 12.1% among users of injectable drugs, sex workers, and MSM, respectively\(^11\)\(^-\)\(^13\). These prevalence rates are 12, 15, and almost 30 times larger than in the general population. Groups at major risk of infection from HIV in Brazil play an important role in the spread of the epidemic\(^14\), and there is international evidence that interventions focused on high-risk groups have an impact on the incidence of HIV in the general population\(^15\).

Current information about infection, behaviors and programs are essential for understanding the dynamics of the HIV epidemic and improving programs. In response, the Department of Sexually Transmitted Infections and Viral Hepatitis (DIAHV) supported a second national round of Biological and Behavioral Surveillance Survey (BBSS) among MSM in 2016 using Respondent-driven Sampling (RDS). RDS is a chain-link sampling method that permits participants to recruit their acquaintances and uses a mathematical model to adjust for this method of recruitment\(^16\)\(^-\)\(^18\). RDS is used in hundreds of surveys\(^19\)\(^-\)\(^21\) around the world and was recently identified as a major contribution to social, behavioral and economic research by the U.S. National Academies of Sciences\(^22\).
RDS data collection is initiated by selecting a small number of initial participants (seeds) from the population of interest. Seeds are extensively briefed about the purpose and operation of the study, and the information to be provided recruiters. They initiate recruitment with a fixed number of coupons (often three) to provide to the participants recruited. When these recruits complete the survey, they are provided with coupons and both themselves and their recruiter receive incentives\textsuperscript{16,23,24}. The fact that participants are recruited by their peers contributes to the success of RDS. Additionally, bias associated with interviewers selecting participants is avoided\textsuperscript{16,23,24}. RDS requires researchers to understand some of the underlying networks of the population being recruited, to assess if seed selection unduly influenced the final sample, and to identify recruitment bottlenecks and missed subpopulations. Gile et al. do an excellent job describing these issues and recommending diagnostics\textsuperscript{25}. Our paper describes the methods used to conduct the BBSS for HIV among MSM in 2016 and applies several diagnostic tools. This paper is STROBE-RDS compliant\textsuperscript{26}.

\section*{MATERIALS AND METHODS}

The study took place in 12 Brazilian municipalities in the five regions of Brazil from June to December 2016: Manaus, Belém (North Region); Fortaleza, Recife, Salvador (Northeast Region); Brasília, Campo Grande (Central-West Region); Belo Horizonte, Rio de Janeiro, and São Paulo (Southeast Region); and Curitiba, Porto Alegre (South Region) (Figure 1).

All of the surveys were conducted in public health facilities except in Belo Horizonte and Salvador where private offices were used. Study working hours were adjusted to include evenings or weekends as required. The study sites consisted of a receptionist and waiting area, interview areas, and a private room for testing and counseling.

\section*{STUDY POPULATION}

Our sample included men, 18 years of age or older, reporting oral or anal sex with another man or transgender woman (travesti) in the last 12 months; and living, studying, or working in the studied city. Respondents were asked about their relationship to the person providing the coupon. The questionnaire and biological testing were consented separately, and participants could opt out of testing. Individuals under the influence of drugs or alcohol, or who identified as a transgender woman, were excluded.

\section*{SAMPLE SIZE}

RDS requires a design effect (DE) multiplier. DE is the ratio of the actual variance to the variance expected with simple random sampling. Wejnert et al. recommend
a DE of 4\textsuperscript{27}. Using this recommendation and HIV prevalence by city from previous studies we calculated sample sizes for each city: Belém (393), Manaus (204), Salvador (193) Fortaleza (131), Recife (116), Brasília (299), Campo Grande (143), Belo Horizonte (267), Rio de Janeiro (450), São Paulo (474), Porto Alegre (393), and Curitiba (210). Donors limited sample size to 350 per city, which complies with a DE of 2. Most RDS studies reach convergence at wave 5 or 6, much earlier than the sample size of 350 participants.

FORMATIVE RESEARCH

Before initiation of the BBSS we conducted formative research (FR) as recommended\textsuperscript{28} using both individual interviews and Focus Group Discussions (FGD)\textsuperscript{29}. The individual and FGD interview guides covered sexual identities, social and geographic organization of MSM in each city and perceived community acceptance, including violence, homophobia and stigma. Questions related to study logistics such as the site of the study, hours

Figure 1. States and cities where respondent-driven sampling (RDS) among men who have sex with men (MSM) was conducted in 2016, Brazil.
of operation, social network size questions, willingness to participate and to test in the study and potential to serve as seeds were explored until saturation of responses for that topic was achieved.

Data collection for the formative research was conducted between December 2015 and March, 2016. FR was conducted by a team consisting of the national and site coordinators, and a qualitative research expert to assure uniformity. Interviews for both individual and FGDs were recorded but not transcribed. Initial interview notes were expanded by reviewing the recordings.

A convenience sample of participants was recruited by Site Coordinators from local non-governmental organizations (NGOs) that work with MSM and from voluntary HIV testing and counseling centers. We conducted 58 one-on-one interviews and 17 FGDs. A total of 184 MSM formally participated in the FR. Overall enthusiasm for the FR and BBSS was high. The Project logo, Me Convida que Eu Vou (Invite me so that I will go) was also endorsed in the FR. Site coordinators were instructed to continue documenting community response and other issues during the study.

BIOLOGICAL AND BEHAVIORAL SURVEILLANCE SURVEY DATA COLLECTION

Five to six seeds were identified for each site. After completing the survey (interview and testing), each seed received three non-reproducible coupons to distribute. Each eligible participant completing the survey, including the seeds, received R$ 25 » US$10. Each participant was also eligible to receive an additional R$ 25 for each recruitee that completed the survey. When approximately 75% of the sample had been recruited, the instructions to recruiters emphasized that when sample size was reached coupons would not be redeemable. In some sites one or two seeds were added when the directors were concerned about the speed of recruitment (Table 1). Seeds and recruiters were trained to provide a detailed explanation of the study to their recruits. The need to recruit participants that met eligibility requirements from their personal networks was emphasized. If the participant allowed, telephone messaging was used to remind respondents of appointments and to contact recruiters if their recruitees did not show up.

Arriving at the site, eligibility of the recruit was reviewed. Reasons for non-eligibility were explained and HIV educational materials and condoms given. Coupons and IDs were managed with an on-line coupon generator. The recruit was then read a description of the study, and risks and benefits of participation. After consenting, an interviewer using Computer Assisted Self Interview (CAPI) administered the social network size questionnaire. The social network question cascade (4 questions) is summarized in the following two questions: “How many men do you know and who also know you, who have sex with other men (oral or anal) in the last 12 months, live, study and/or work in [municipality], are 18 years old or older, you encountered or spoke with in the last two months? Of these how many would you invite to participate in this study?”
Table 1. 2016 RDS survey duration, seeds and longest wave, and eligibility by site (sample size n = 4,176).

| Site            | Manaus | Belém | Fortaleza | Recife | Salvador | Campo Grande | Brasília | Belo Horizonte | São Paulo | Rio de Janeiro | Curitiba | Porto Alegre |
|-----------------|--------|-------|-----------|--------|----------|--------------|----------|----------------|-----------|----------------|----------|--------------|
| Start (2016)    | 07/12  | 07/12 | 07/11     | 07/12  | 07/26    | 07/14        | 09/13    | 06/22          | 06/23    | 08/01          | 07/21    | 08/22        |
| Total Weeks     | 7.5    | 5.9   | 9.6       | 6.9    | 9.2      | 8.7          | 11.3     | 7.7            | 9.7      | 17.6           | 13.6     | 14.6         |
| Total Seeds     | 7      | 6     | 5         | 7      | 5        | 6            | 6        | 6              | 6        | 5              | 6        | 6            |
| Longest Waves   | 12     | 8     | 10        | 18     | 14       | 15           | 13       | 14             | 13       | 15             | 21       | 14           |
| Ineligible      | 19     | 4     | 1         | 4      | 4        | 2            | 4        | 25             | 9        | 27             | 22       | 3            |
| Reasons         |        |       |           |        |          |              |          |                |          |                |          |              |
| < 18 yrs        | 3      | 0     | 0         | 1      | 1        | 2            | 1        | 2              | 0        | 1              | 4        | 1            |
| Not local       | 1      | 2     | 0         | 0      | 0        | 0            | 0        | 1              | 5        | 1              | 3        | 1            |
| No Sex < 12 m   | 13     | 2     | 0         | 2      | 3        | 0            | 0        | 5              | 3        | 22             | 12       | 0            |
| Incapable       | 0      | 0     | 0         | 1      | 0        | 0            | 1        | 0              | 0        | 0              | 0        | 0            |
| Total eligible  | 351    | 350   | 356       | 359    | 350      | 352          | 361      | 350            | 353      | 325            | 352      | 338          |
| Sample Size     | 351    | 350   | 353       | 349    | 350      | 352          | 359      | 350            | 351      | 325            | 348      | 338          |
| Network (median – min. – max.) | **6** (1 – 200) | **5** (1 – 1,000) | **4** (1 – 687) | **8** (1 – 250) | **10** (1 – 300) | **10** (1 – 5,000) | **10** (1 – 300) | **11** (1 – 1,000) | **10** (1 – 2,500) | **10** (1 – 2,500) | **7** (1 – 380) | **10** (1 – 270) |
| % coupons returned | 33.0   | 33.0  | 33.0      | 33.0   | 33.0     | 33.0         | 33.0     | 33.0           | 33.0     | 33.0           | 33.0     | 33.0         |
Following completion of the questionnaire, the respondent was consented separately for each test and sent for testing.

Following counseling, two tubes of venous blood were drawn. For HIV, blood was first tested with the rapid test RT1 Anti-HIV (Alere/Bioeasy). If positive, the blood was tested with RT2 HIV (Abon). Two positive results fulfilled Ministry criteria for reporting HIV+ serostatus. Respondents were counseled and immediately referred if positive. For syphilis, blood was tested with RT Anti-Syphilis (Biomanguinhos). For syphilis and all positive tests, results were explained to respondents and they were referred for counseling and treatment. Remaining rapid tests included RT Hepatitis B – HBsAg (Vikia) RT Hepatitis C — Anti-HCV (Alere). After the rapid tests, the tubes were centrifuged and stored at -20ºC. Samples were sent to the national reference laboratory — Instituto Adolfo Lutz, São Paulo — for confirmatory testing. Transport complied with requirements of the Agência Nacional de Vigilância Sanitária (ANVISA).

**TRAINING**

A series of four workshops about RDS was held: introduction to RDS, organization of the study, data analysis, and write-up. Organization of the study was a 3-day workshop for site coordinators who then trained their local teams. Following the workshop, a pilot implementation was then conducted in each site. After this exercise, the teams met via videoconference to identify issues. Videoconferences were held repeatedly with sites until concerns were successfully addressed. When necessary, sites were visited in person. All study procedures including scheduling interviews, tablet use, pre-test counseling, referral, post-test counseling, coupon generation and incentive management were documented in standard operating procedure (SOP) manuals, provided to and approved by the DIAHV before initiation of fieldwork.

**DATA COLLECTION INSTRUMENT**

The questionnaire was adapted from the 2009 BBSS questionnaire used in Brazil and contained items to report international AIDS indicators for Brazil and other questions related to the national program. The questionnaire was modified to:

- improve comparison with items in the 2009 survey;
- collect information related to policy and program changes (e.g., PrEP and PEP) and to environments and HIV-linked behaviors in the MSM population.

The questionnaire was organized into blocks:

- identification and eligibility;
- social network;
- socioeconomic and demographic information;
- sexually transmitted infections, HIV/AIDS and hepatitis B and C;
- access to health services in general and specifically for HIV prevention and treatment;
- history of HIV, syphilis and hepatitis testing;
- information about HIV/AIDS, PEP, PrEP and treatment for HIV/AIDS;
- gay and homosexual visibility;
- violence, stigma and discrimination;
- sexual behavior including condom use;
- drug and alcohol use;
- social inclusion and participation;
- mental health.

**DATA MANAGEMENT**

Each site was equipped with high-speed and reliable internet provided by the project. Information entered from computer or tablet was encrypted, uploaded and stored on the project website. The website was maintained by an Information Technology professional and copies of the database archived on the project server and on the PI’s own desktop computer stored in a locked office in Fortaleza. The reference laboratory stored their data locally and on mirrored backups offsite.

**ETHICAL CONSIDERATIONS**

The overall study was approved by the Committee on Research Ethics of the Federal University of Ceará (UFC), credentialed by the National Commission on Research. As mentioned above, all participants signed a consent form to participate in the interview and separately consented for each test that was offered. There appeared to be little reluctance to participate in both parts of the study, confirming results obtained from formative research.

**ANALYSIS**

Analysis proceeded as follows. Gile’s successive sampling (SS) estimator\(^9\) was used to produce weighted estimates using RDS Analyst version 1.7-16. The estimator assumes a finite population and requires a population size estimate for each sample. To calculate this, we used the proportion of men who self-reported as having had at least one same-sex relationship in the National Survey of Knowledge, Attitudes and Practices in the Brazilian population (18 – 64) conducted in 2013\(^1\). This survey was powered to provide regional estimates. We applied this regional estimate to the total male population 18 – 64 in each
city of that region as provided by the Brazilian Institute of Geography and Statistics. To test the accuracy of this procedure, we compared the percentage of MSM population calculated with this method in São Paulo city with the percentage obtained from a separate municipal survey conducted in São Paulo that used the same sampling methodology as the national survey, powered for city estimates. Both results (3.9 and 3.6%) were not significantly different.

To provide a national estimate, we merged the 12 cities to create a single dataset. We used the Complex Analysis Survey tools in Stata 14.0 with each city treated as its own stratum to weight the final results.

**RESULTS**

A total of 4,176 respondents was recruited (Table 1). Some summary sociodemographic details of the final sample are presented in Table 2.

**DIAGNOSIS AND STUDY ISSUES**

Diagnostic criteria for RDS includes simple inspection of recruitment data as well as tools provided by RDS Analyst. As proposed in Gile et al., we reviewed reported personal network sizes for consistency and reasonableness. Additional tools include convergence and bottleneck plots. Convergence — a stable estimate of the true population proportion — should be achieved rapidly for major variables. Bottleneck plots visually demonstrate convergence by recruitment chain: widely different indicator estimates by chain would signal important differences by seed and a failure to find a true population proportion. In a non-technical sense, it demonstrates if a recruitment chain is ‘stuck’ in a sub-population or geographical region. Review of convergence and bottlenecks is through visual inspection and interpretation (Table 1 and Figure 2). The convergence plot shows when in recruitment the outcome estimate is determined. The bottleneck plot shows the estimate in terms of each recruitment chain. Visual inspection can show if one or several chains demonstrate different outcome values. Given 5 seeds, an ideal bottleneck plot would stabilize after a few waves producing a single horizontal line.

Data were collected over a 4-month period, much faster than recruitment in 2009 and in many other surveys. Data collection times varied between 5.9 and 17.6 weeks. The surveys in Rio de Janeiro and Porto Alegre failed to achieve their sample size due to the late start of data collection in reason of a six-month delay in local IRB approval. Median network size reported (4 – 10) was both relatively small and uniform across sites, which is a positive sign. The length of the longest wave varied from 8 in Belém to 21 in Curitiba (Table 1). Convergence was achieved on major variables for all sites. We illustrate this
with HIV serostatus (Figure 2) which presents results for selected cities in the 5 regions. While there was reason to assume from the formative research that there might be bottlenecks that interfered with completion of the survey due to age, identity, behavior and

Table 2. Socioeconomic and demographic characteristics of participants in 12 cities.

|                                | %   | 95%CI        |
|--------------------------------|-----|--------------|
|                                |     | L          | U       |
| Age (years)                    |     |            |         |
| < 25                           | 58.3| 54.6        | 62.0    |
| ≥ 25                           | 41.7| 38.0        | 45.4    |
| Race                           |     |            |         |
| White                          | 31.8| 28.5        | 35.3    |
| Black                          | 21.8| 19.0        | 25.0    |
| Amarela (Asian descent)        | 2.4 | 1.6         | 3.7     |
| Parda (Mixed)                  | 42.0| 38.5        | 45.6    |
| Indigenous                     | 1.9 | 1.1         | 3.3     |
| Years of School (years)        |     |            |         |
| ≤ 4                            | 3.5 | 2.1         | 5.6     |
| 5 – 8                          | 9.4 | 7.6         | 11.6    |
| 9 to 11                        | 16.8| 14.3        | 19.5    |
| High school/Incomplete college | 59.3| 55.7        | 62.8    |
| Complete College               | 11.1| 9.0         | 13.6    |
| Socioeconomic Strata           |     |            |         |
| A/B (higher)                   | 40.7| 37.3        | 44.2    |
| C (middle)                     | 43.0| 39.4        | 46.7    |
| D/E (lower)                    | 16.2| 13.8        | 19.0    |
| Civil Status                   |     |            |         |
| Single                         | 83.0| 80.1        | 85.6    |
| Married                        | 3.6 | 2.5         | 5.2     |
| Same sex stable union          | 10.2| 8.1         | 12.7    |
| Stable union with woman        | 1.0 | 0.5         | 2.0     |
| Separate/divorced              | 2.0 | 1.4         | 3.0     |
| Widower                        | 0.3 | 0.1         | 0.7     |
The 12 City HIV Surveillance Survey Among MSM in Brazil 2016 Using Respondent-Driven Sampling: A Description of Methods and RDS Diagnostics

| Plot     | Convergence | Bottleneck |
|----------|-------------|------------|
| Belém    | ![Convergence plot of HIV4 = 1](image) | ![HIV4 = 1](image) |
| Fortaleza| ![Convergence plot of HIV4 = 1](image) | ![HIV4 = 1](image) |
| Brasília | ![Convergence plot of HIV4 = 1](image) | ![HIV4 = 1](image) |

Figure 2. Bottleneck and convergence for HIV by city in Brazil (respondent-driven sampling – RDS – among men who have sex with men – MSM – 2016).
| Plot          | Convergence                          | Bottleneck                           |
|--------------|--------------------------------------|--------------------------------------|
|              | ![Convergence plot of HIV4 = 1](#) | ![HIV4 = 1](#)                       |
| Rio de Janeiro | ![Estimated HIV4 = 1](#)            | ![Seed](#)                           |
|              | 0.00 0.25 0.50 0.75 1.00            | 1 2 3 4 5 6                          |
|              | # of Observations                    | # of Observations                     |
| Curitiba     | ![Convergence plot of HIV4 = 1](#) | ![Seed](#)                           |
|              | 0.00 0.25 0.50 0.75 1.00            | 1 2 3 4 5 6                          |
|              | # of Observations                    | # of Observations                     |
| Porto Alegre | ![Convergence plot of HIV4 = 1](#) | ![Seed](#)                           |
|              | 0.00 0.25 0.50 0.75 1.00            | 1 2 3 4 5 6                          |
|              | # of Observations                    | # of Observations                     |

Figure 2. Continuation.
class, it did not appear to be the case. One site, Porto Alegre, that might have required a larger sample size, achieved convergence, and the recruitment chains do not demonstrate bottlenecks (Figure 2).

Two sites, Rio de Janeiro and São Paulo (not shown), that required sample sizes larger than 350, did show a delayed convergence. While convergence for HIV prevalence in Rio de Janeiro ultimately appeared stable, early results along with the bottleneck chart demonstrate wide differences and separation in the recruitment chains in the early weeks of the study.

LIMITATIONS

The study reported here suffers from some limitations. RDS, required for comparison to previous national estimates, remains controversial. Limitations inherent in RDS are well reviewed elsewhere. Specific cities and sample size were determined by the donor. This may have affected our results in the cities where our calculations required a larger sample size. However, in São Paulo our results are not statistically different from a previous study conducted in the city using Time Location Sampling. The 2016 study took place at a time of both great political change in Brazil and with an HIV program focused on treatment. Both changes have led to a disengagement with MSM — and MSM serving organizations with potential effects on participation and the survey.

DISCUSSION

Overall, we argue that the study was successful: large sample sizes were achieved in a relatively short period of time, with no evidence of difficulties with convergence and little evidence of bottlenecks in recruitment. One important reason is overall receptivity among MSM in our sample. This happened in spite of the changes in national support reported in limitations. Many NGOs that participated in the 2009 study had closed by 2016. In half of the cities, finding MSM organization partners for the study was initially difficult. Perhaps these closures served to motivate participants, but we have no direct evidence of that. In the FR, enthusiasm for the study appears to be associated with an enhanced willingness to test for HIV and other infections in studies directed to MSM. Many studies report health disparities and barriers to health care for MSM. Willingness to participate in our study may also be associated with a reaction to these barriers and an understanding of the importance of these outcomes to direct resources to MSM. In our study 90.2% of participants tested. The formative research exercise itself may also have encouraged participation. Finally, the rapid completion of the study and its quality may be associated with the experience the research team had with the method from the earlier RDS study.
One issue influencing the conduct of the study was the delay in the IRB approval process. In our study, using an almost entirely standardized instrument and testing procedures preapproved by national authorities, with review and approval by a national IRB, several local IRBs, rather than defer, demanded separate reviews, extending the project start by six months in those locations. As the pace of scientific innovation (and demands on local expertise) increases, the role of local IRBs in national studies needs to be reviewed. Routinized surveillance is especially vulnerable to localized responses; much can be hidden by simply not collecting the data.

Finally, there is some concern in the research community for reproducibility of RDS\textsuperscript{34}. It is a concern for the reliability of RDS and its lack challenges RDS as a valid sampling method. BBSS is also meant to be repeated regularly to monitor prevalence and program. If results from two survey rounds are different, can we attribute that to changes in study outcomes, characteristics of the study, or RDS itself? There are many potential answers to these questions, but certainly one would be to use diagnostic tools and formative research to assist interpretation and comparison. At least for surveillance, health authorities and community groups familiar with these tools are the best arbiters of what surveillance data means.

**CONCLUSIONS**

This paper reports on the methods used in the most recent national BBSS for MSM in Brazil. The comprehensive BBSS was completed as planned and on budget. The description of methods is more detailed than usual, due to new diagnostic tools and requirements of the new STROBE-RDS guidelines\textsuperscript{26}. Because RDS requires strong statistical assumptions, these reports are increasingly important, both to assist in interpretation of findings and to encourage continued development of RDS. Reliable and precise estimates of HIV and other diseases in key populations over time are required if the true scope of the global HIV pandemic and conditions of vulnerability created by disparities is to be addressed.

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