# Letter to the Editor



# Estimating HIV Incidence Rates among MSM in an Urban Area of Chongqing Using Three Approaches<sup>\*</sup>

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To evaluate the HIV pandemic in Chongqing, pooled PCR, lgG-capture BED the enzyme immunoassay (BED-CEIA), and cohort observations were used to estimate the HIV incidences among men who have sex with men (MSM). 617 MSM subjects completed the survey at a voluntary counseling and testing (VCT) site. The observed HIV incidence was 12.5 per 100 P-Ys (95% CI = 9.1-15.7). The annual acute HIV infection (AHI) incidence estimated by pooled PCR was 14.0% (95% CI = 10.9-17.1). The HIV-1 annual incidence estimated based on the BED-CEIA was 12.0% (95% CI = 7.5-16.5). The HIV incidences estimated by these three approaches were consistent and complementary. The HIV incidence rates were alarmingly high with an uptrend among the urban MSM of Chongqing.

The incidence of HIV has been identified as the most precise quantitative indicator of the extent of ongoing HIV transmission and also the most reliable indicator of the impact of HIV prevention measures in a given population<sup>[1]</sup>. However, HIV is a chronic infection, and recently reported cases may include patients who were infected before the notification.

During the past few years, there have been increasing concerns regarding the worldwide resurgence of HIV infection among men who have sex with men (MSM). In Chongqing, with a total of 31,922 HIV/AIDS cases reported in 2016, the prevalence of HIV in the general population exceeds 0.1%. The National Surveillance System reported that Chongqing is ranked fifth among the 31 mainland provinces in China (unpublished data). It is also reported that 20% of the infections were caused by homogeneous contacts (this result may have

been underestimated due to stigma or discrimination).

HIV incidence has been traditionally observed in prospective cohorts. However, it is difficult to establish HIV incidence in a MSM cohort. Over the past few years, numerous laboratory approaches have been used to estimate HIV incidence based on surveys conducted in cross-sectional populations. lgG-capture BED enzyme immunoassay The (BED-CEIA), initiated by the US Centers for Disease and Prevention (CDC) was Control widely implemented in China between 2005 and 2014 to determine HIV incidence in this resource-limited country. However, reported misclassification limited its widespread use for routine surveillance. Proposed by Brookmeyer and Quin, pooling of serum or plasma for HIV-1 RNA in antibody-negative individuals with early infection has been used to improve the accuracy<sup>[2-4]</sup>.

This study was conducted to estimate HIV incidence among MSM *via* three approaches to research their efficiency.

Between February and July of 2008, a total of 617 male volunteers were recruited for this study via respondent-driven sampling at voluntary а counseling and testing (VCT) clinic located at a transportation-convenient site administered by the Chongqing CDC. Subjects were aged  $\geq$  18 years, have resided in Chongqing for > 6 months, and claimed to have had oral or anal sex with another man during months. the past 6 Questionnaires were administered to collect demographic and behavioral data. All subjects provided informed consent prior to HIV testing, and the study was approved by the Ethics Committee of Chongqing CDC.

doi: 10.3967/bes2017.122

<sup>&</sup>lt;sup>This</sup> study was supported by the Department of Health and Family Counseling, Chongqing [2016HBRC009, 2017ZDXM001].

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Each specimen was screened at the clinic using a rapid test kit (Alere Determine HIV-1/2, Alere Medical Co., Ltd., Matsudo-shi, Japan). All samples were submitted to an antibody confirmatory laboratory due to the sensitivity- and specificity-associated issues that might arise during rapid HIV testing. A 4<sup>th</sup>-generation antibody and p24 antigen detection ELISA kit (BioMerieux, the Netherlands) was used for the repeated screening. Western blot (MP Biomedical Asia Pacific Pte. Ltd., Singapore) was performed to confirm antibody test positivity.

Specimens that were positive according to ELISA but negative according to Western blot were included for the pooled nucleic acid amplification testing (NAAT) (Amplicor HIV-1 Monitor 1.5 assay; Roche Molecular Systems, Somerville, NJ, USA). Specimens that were antibody-positive according to Western blot were tested to identify recent HIV infection (RHI) by BED-CEIA (Calypte Biomedical Corporation, Portland, OR, USA). The experimental data were managed using an Excel spreadsheet designed by the US CDC.

Individuals who were HIV antibody-negative were enrolled in the prospective cohort and followed-up for approximately 6 months. The participants were required to return to the site for HIV antibody screening tests 6 months later.

HIV incidence within a cohort was expressed as the number of new infections per person-year (P-Y), whereas the BED cross-sectional estimate utilized an 'at-risk' formula<sup>[5]</sup>. The incidence rate determined by the pooled PCR reflected the number of AHI cases. The crude data was entered in the EpiData 3.02 software (The EpiData Association Odense, Denmark) and analyzed using SAS software version 9.1 (SAS Institute Inc. Cary, NC, USA).

Formula for cohort incidence:

$$I = \frac{N_{\text{pos}} \times 365}{(N_{\text{neg}} - N) \times T} \times 100\%$$

Formula for BED-CEIA incidence<sup>[5-6]</sup>:

$$I = \frac{\binom{365}{W} \times N_{\text{inc}}}{N_{\text{neg}} + \binom{365}{W} \times N_{\text{inc}}} \times 100\%$$

Formula for pooled PCR incidence<sup>[7-8]</sup>:  $I[1-(M/N)^{1/c}] \times 365/\mu \times 100\%$ 

According to the demographic analysis, the recruited subjects covered almost all urban districts of Chongqing municipality. The mean age of the subjects was  $25.6 \pm 6.3$  years, and 73.8% were local residents. Of the participants, 97.7% were of Han

ethnicity; additionally, 85% were single, 8.3% were married, and 6.3% were divorced. Overall, 70.1% of the participants had received a college education, 27.6% were in the process of receiving a college education, and 26.6% were business company employees. Of the study subjects, 70.6% claimed that they were homosexual, and 29.4% claimed that they were bisexual. A total of 52.4% participants reported having unprotected anal intercourse during the past 6 months. The mean age at the unprotected anal sex debut was 19.8 ± 3.6 years, and the minimum age was 10 years. The average number of male partners was 4.8 ± 17.9. Of the subjects, 57.9% were unaware of the infection status of their sex partners, whereas only 3.0% reported having been informed of the infection status of their partner.

According to serological results, 129 participants were HIV antibody-positive. After the positive subjects were excluded, 488 participants were enrolled in the cohort, and 214 (43.9%) were followed-up for 6 months. The total accumulated observation person time was 107 P-Ys. According to the results of the multivariate logistic regression analysis, education and syphilis were independent risk factors for HIV seroconversion among MSM.

Five samples were positive according to pooled NAAT, indicating that these subjects had AHIs. Thus, the baseline prevalence of HIV among MSM in Chongqing in 2008 was 20.9% (95% CI = 14.1-27.7). In accordance with the WHO recommendations for the BED incidence estimation<sup>[5]</sup>, we excluded specimens with low CD4 counts, specimens derived from subjects with long-term infections according to the case reporting system, and specimens that were unqualified. In total, of 123 antibody-positive specimens tested by BED-CEIA, 27 were RHIs. During the follow-up period, a total of 14 MSM converted to HIV antibody-positive. The AHI incidence derived via pooled PCR was 14.0% (95% CI = 10.9-17.1), whereas an incidence of 12.5 cases per 100 P-Ys (95% CI = 9.1-15.7) was observed in the longitudinal cohort. The incidence rate estimated via BED-CEIA was 12.0% (95% CI = 7.5-16.5). Thus, there was some agreement between the estimates, as indicated by the overlapping  $Cls^{[3]}$  (Table 1).

The subgroup containing MSM aged 25-34 years had the highest incidence of AHI at 29.9% (95% CI =23.4-36.3), and an AHI incidence rate of 6.0% (95% CI= 2.8-9.2) was identified in MSM aged < 25 years. Participants aged  $\geq$  34 years demonstrated the lowest AHI incidence at zero. However, the highest incidence rates estimated using BED-CEIA and observed

			Tab	l <b>e 1.</b> Incic	lence Estimate	d Using Diff	erent Methods			
		Poole	ed PCR		BI	ED-CEIA			Cohort	
Subgroups	NO. Tested	NO. of AHIs	Incidence (95% <i>C</i> I)	Total NO.	NO. Seropositive	NO. of RHIs	Incidence (95% <i>CI</i> )	NO. Recruited	NO. Seroconverted	Incidence (95% <i>CI</i> )
By age										
Total	488	ъ	14.0 (10.9-17.1)	617	123	27	12.0 (7.5-16.5)	488	14	12.5 (9.1-15.7)
Under 25 years	220	1	6.0 (2.8-9.2)	278	49	16	14.6 (7.5-21.8)	220	6	18.3 (12.9-23.1)
25-34 years	193	4	29.9 (23.4-36.3)	244	53	10	11.3 (4.3-18.4)	193	4	9.2 (5.1-13.2)
34 years and older	75	0	0	95	27	1	3.6 (-3.4-10.6)	75	1	5.9 (0.5-11.2)
By residence										
Local	360	ŝ	11.3 (8.0-14.5)	455	96	18	10.8 (5.8-15.8)	360	10	12.6 (8.6-15.3)
Migrant	128	2	21.9 (14.8-29.1)	161	33	6	15.4 (5.3-25.5)	128	4	14.2 (7.9-20.0)
By age and residence										
Local aged 25-34 years	142	2	19.6 (13.1-26.1)	180	36	8	11.3 (3.5-19.3)	142	2	6.4 (2.4-10.4)

within the cohort among MSM aged < 25 years were 14.6% (95% *Cl* = 7.5-21.8) and 18.3 per 100 P-Ys (95% *Cl* = 12.9-23.1), respectively. The oldest subgroup, which contained participants aged  $\geq$  34 years, had the lowest HIV incidence at 3.6% (95% *Cl* = -3.5-10.7) but the highest prevalence at 28.4% (95% *Cl* = 19.3-37.4) based on the results of the baseline BED assay; this finding was supported by both the pooled PCR estimates and the cohort observations.

Moreover. the residence-stratified rates estimated using the three approaches suggested that HIV incidence was higher among the migrant population than among the local MSM population. The AHI incidence among the local MSM population was 11.3% (95% Cl = 8.0-14.5), whereas it was 21.9% (95% Cl = 14.8-29.1) among the migrant MSM population. The RHI incidence indicated by BED-CEIA was 10.8% (95% CI = 5.8-15.8) among the local MSM population, whereas it was 15.4% (95% *Cl* = 5.3-25.5) among the migrant MSM population. The incidence rates observed within the cohort were 12.6 per 100 P-Y (95% CI = 8.6-15.3) and 14.2 per 100 P-Y (95% CI = 7.9-20.0) among the local and migrant MSM populations, respectively (Table 1).

In our study, we estimated true HIV incidence rates among the MSM population in Chongqing via pooled PCR tests, BED-CEIA, and prospective observations within a cohort. The features of different approaches, particularly the time point at which biomarkers may appear, are worth additional consideration. Pooled PCR, which is performed to identify positive specimens, has a very short window of 28 days<sup>[8]</sup> to reveal the acute infection rate. This rate may serve as an indicator of earlier epidemic stages within an area or at-risk population and may be an important complement to the BED results. We concluded that the AHI incidence rates obtained via pooled PCR and RHI obtained via BED strongly each other. Moreover, the support cohort observation, which was performed to identify subjects in whom antibodies were being converted, indicated HIV incidence 180 days later than the pooled PCR. This discrepancy occurs because patients take an average of 6 months to completely convert from antibody-negative to positive. The cohort result confirmed the uptrend in the HIV epidemic among MSM. The result of the BED assay revealed the incidence at the time point when the cross-sectional baseline survey was administered, and thus, this data disclosed the true stage of the epidemic approximately 6 months earlier than the

cohort, as the period for this test is 155 days<sup>[9]</sup> in China. However, the results derived using the aforementioned three methods were correlative and in accordance because all results were converted to annual incidence rates.

According to demographic and behavioral data obtained from the questionnaire, MSM in Chongqing continued to engage in risky behavior. When considered in combination with the incidence rates identified herein, these data are highly indicative of the critical epidemiological situation of HIV in MSM in Chongqing urban area. The high rate of unprotected anal intercourse (52.4%), young age at unprotected anal sex debut (19.8  $\pm$  3.6 years), high number of male partners (4.8  $\pm$  17.9), and lack of awareness of the HIV infection status of their sex partners (only 3.0% had been clearly informed) were factors identified in this population that might have substantially contributed to the ongoing epidemic.

However, the low retention rate observed in the cohort during the 6-month follow-up period should not be ignored due to which alternative approaches such as BED and pooled PCR should be given more concern.

Although the data in our study was collected in 2008, it revealed a previous regional HIV epidemic status among MSM and was more referential to peers majoring in HIV incidence evaluation presently. Scientists from the US CDC and the WHO prefer using the Limiting Antigen (LAg) Avidity enzyme immunoassay, which has been more widely applied than BED in China since 2015. Nevertheless, after excluding specimens with low CD4 counts, BED in our study did not appear to overestimate the incidence as reported.

We strongly recommend further analysis based on subgroups of age, gender, residence, and behavior information, as long as the sample sizes are sufficiently large.

In summary, HIV incidence rates among MSM in Chongqing determined using pooled PCR, BED-CEIA, and cohort observations verified the highly concordant epidemic uptrend. The laboratory incidence evaluation approaches and epidemic observation approach were strongly supportive of each other.

The HIV-1 incidence rates were alarmingly high among MSM in the urban area of Chongqing, particularly within subjects aged < 25 years and migrants aged 25-34 years.

The authors would like to thank Dr. Zhao JK,

who works in the Global Fund to Fight AIDS, Tuberculosis and Malaria, and Dr. Zheng DP, who works at the US CDC, for their assistance with the survey design and data analysis. The authors would also like to thank the following staff members at the Chongqing CDC for their assistance with participant enrollment and experiments: Zheng JQ, Ou YL, Lu RR, and Ling H. Finally, we are grateful to all participants of the MSM cohort.

The furth details are in the APPENDIX in the website www.besjournal.com

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Received: June 27, 2017; Accepted: November 7, 2017

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### APPENDIX

### Comparison of the costs for the RHI incidence estimation and AHI detection

We used commercial test kits for both the pooled PCR assay and the BED-CEIA. The total costs were approximately US\$8621 and US\$1249, respectively. The results of our study suggested that the AHI prevalence was 1.02% as determined by the pooled PCR; thus, the detection of each individual with AHI cost US\$1724. Considering the large window of the BED-CEIA, the detection of each RHI cost US\$54.30, as shown in Supplemental Table 1. Data regarding the costs required for testing individuals in the prospective cohort setting and maintenance-associated costs were not provided by our colleagues.

Method	No. of Samples	No. of AHI or RI	Control Factor	Repeated Assay Factor	Price per Test (US\$)	Total Tests	Total Expense (US\$)	Cost for the Detection of Each AHI or RI (US\$)
Pooled PCR	488	5	24/19	1.1	73	80	8,621	1724
BED-CEIA	617	23	96/85	1.1	4.41	228	1,249	54.30

Supplemental Table 1. Cost of HIV Incidence Estimations Using Two Laboratory Methods

Control factor 1 = 24/19. The pooledsample PCR tests required the use of 5 control samples for each of the 19 clinical specimens, including the high positive control, low positive control, and negative control samples provided by the test kit and another 2 positive control samples provided by the laboratory.

Total tests: Eightypooledsample PCR tests were performed in our study, including 5 stage-I pools, 25 stage-II pools and 50 stage-III pools. The 5 stage-I pools tested positive, and thus every positive stage-I pool was subdivided into 5 stage-II pools; accordingly, a total of 25 stage-II pools were tested. Next, 25 stage-II pools tested positive and were subdivided into 50 stage-III pools. For the BED, 123 positive specimens were screened, of which 35 specimen required triple confirmation test according to the standard operation procedure.

Control factor 2 = 96/85. The BED assays required the use of 11 control samples for each of the 85 clinical specimens, including the high positive control, low positive control, negative control, and calibrator samples provided by the test kit.

Repeated-assay factor: Since assay failures may occur, a repeated assay factor of 1.1 was estimated based on the laboratory staff's experience.

Price per test (US\$) referred to current market prices.