IS THE BED CAPTURE ENZYME IMMUNOASSAY USEFUL FOR SURVEILLANCE IN CONCENTRATED EPIDEMICS? THE CASE OF FEMALE SEX WORKERS IN INDONESIA

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Abstract. Although the BED capture enzyme immunoassay (BED-CEIA) tends to over-estimate HIV incidence in general population epidemics, its limitations may be less relevant to some sub-populations in concentrated epidemics. This study assesses the plausibility of BED-CEIA estimates for female sex workers (FSWs) in Indonesia. Data were derived from a cross-sectional anonymous linked behavioral and biological surveillance survey. Independent samples of 2,917 direct and indirect FSWs, were gathered from seven and five cities, respectively, via three-stage time-location sampling. Participants provided behavioral information, venous blood and vaginal swabs. Specimens testing positive for HIV were subjected to BED-CEIA to identify recent infections.The median duration of sex work was 12 months. The estimated HIV prevalence was 8.2% and the incidence was 4.1 per 100 person years, slightly lower than an Asian Epidemic Model (AEM) estimate. HIV incidence was higher among: direct FSWs (p<0.001), those reporting genital ulcers in the past year (p<0.001), those with active syphilis (p=0.017), and those not receiving periodic presumptive treatment for STIs during the previous 6 months (p=0.045). Low general population HIV prevalence, short durations of sex work and low ART coverage of those eligible for treatment make it unlikely that HIV incidence estimates for FSWs in Indonesia are distorted by long-standing infections and viral suppression. External consistency with model-based estimates and internal consistency in regard to known risk factors for HIV infection add to the plausibility of the estimates. Pending advances in methods for estimating HIV incidence, it may be premature to summarily dismiss the BED-CEIA in concentrated HIV epidemics.

Keywords: BED assay, HIV incidence, concentrated epidemics, female sex-workers, surveillance, Indonesia

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INTRODUCTION

The serologic testing algorithm for recent HIV seroconversion (STARHS) represents a breakthrough in HIV surveillance, allowing for identification of recent HIV infections through re-testing

of specimens reactive to a sensitive HIV test with a less sensitive enzyme immunoassay (Janssen *et al*, 1998; Kothe *et al*, 2003; Rawal *et al*, 2003). The assays first used for this purpose had variable performance for different HIV-1 subtypes, severely limiting their usefulness. The BED capture enzyme immunoassay (BED-CEIA) was formulated with material from HIV-1 subtypes B, E, and D and measures the proportion of HIV-1 specific IgG per total IgG. The BED-CEIA was developed for use across all HIV-1 strains and has been validated using seroconversion panels from multiple HIV sub-types (Parekh *et al*, 2002; Dobbs *et al*, 2004). The BED-CEIA has been utilized worldwide (Hu *et al*, 2003; Barin *et al*, 2005; Buchacz *et al*, 2005; Saphonn *et al*, 2005; Jiang *et al*, 2007; Karita *et al*, 2007; Bärnighausen *et al*, 2008; Mermin *et al*, 2008). However, the accuracy of recent infection estimates obtained with BED-CEIA has been questioned, since they appear excessively high in comparison with either prevalence estimates or with incidences estimated from cohort studies (Karita *et al*, 2007; Bärnighausen *et al*, 2008; Hallett and Garnett, 2009; Lakshmi *et al*, 2009; Todd *et al*, 2009). False incident tests occur when HIV IgG decreases below the test threshold as the result of late-stage HIV disease, immune reconstitution, viral suppression among patients receiving anti-retroviral therapy (ART) and concurrent infections (Killian *et al*, 2006; McDougal *et al*, 2006; Chawla *et al*, 2007; Hargrove *et al*, 2008). About 5% of individuals, fail to progress above the test threshold, and are thus misclassified as recent cases (McDougal *et al*, 2006; Kartikeyan *et al*, 2007; Bärnighausen *et al*, 2008; Hargrove *et al*, 2008). After reviewing the available evidence, UNAIDS recommended the BED-CEIA method not be used for surveillance purposes due to its tendency to overestimate HIV incidence (UNAIDS, 2006).

However, data from men having sex with men (MSM) undergoing frequent, repeated HIV testing in San Francisco (USA) suggest the BED-CEIA accurately measured HIV incidence (Truong *et al*, 2009). The present study assesses whether the BED-CEIA might also produce plausible estimates of HIV incidence in another key population in concentrated HIV epidemics: female sex workers (FSWs) in Indonesia. The HIV strains isolated in Indonesia are mainly sub-type B recombinants and fewer cases of sub-type E (Foley *et al*, 2001; Sahbandar *et al*, 2009).

MATERIALS AND METHODS

The data presented were gathered in the 2007 Integrated Biological-Behavioral Surveillance (IBBS) surveys among most-at-risk groups in Indonesia from independent samples of direct FSWs (*ie*, women who earn their primary livelihood via selling sex) and indirect FSWs (*ie*, women working in massage parlors, karaoke, clubs, bars, etc who sell sex to supplement their income) in seven and five purposively selected cities, respectively, between August and November 2007. Sampling frames consisted of venues where commercial sex was either solicited or took place. These were organized by census block and included an estimated number of FSWs at each venue. Participants were selected through three-stage time-location sampling. Sampling units were selected via systematic-random sampling with a probability proportional to the estimated size during the first and second stages, whereas a fixed number of participants were selected with equal probability during the final stage of sample selection.

Survey field teams were drawn from female staff at the provincial offices of the Central Statistics Bureau and the provincial health offices. Following receipt of witnessed verbal consent, interviewers gathered behavioral information using a structured pre-coded questionnaire. In selected cities, a nurse collected blood through venipuncture, and participants provided self-collected vaginal swabs. The survey and laboratory testing were anonymous, linked via a unique identifier for each participant. Per Indonesian Ministry of Health surveillance guidelines, participants received a coupon for free HIV counseling and testing at a nearby Community Health Center and were given the opportunity to access their STI test results and receive treatment free of charge.

Blood specimens were stored at 4-6ºC to be tested for HIV and syphilis at the nearest government laboratory. HIV was tested using two parallel rapid tests: SD Bioline HIV 1/2 3.0® (Standard Diagnostics, Kyonggi-do, Korea) and Determine® HIV-1 (Abbott, Abbott Park, IL). Discrepant results were re-tested at the national research laboratory using two HIV ELISA tests (enzyme-linked immunosorbent assay): Murex® (Murex Biotech, Dartford, United Kingdom) and Vironostika® (Biomerieux, Marcy l'Étoile, France).

Blood samples were tested for syphilis using rapid plasma reagin (RPR) (Shield Diagnostics, Dorset, United Kingdom), and Determine Syphilis *T. pallidum* (Abbott, Abbott Park, IL) was used as a confirmatory test. RPR results with a titer > 1:8 and TPHA-positives were classified as having active syphilis. Chlamydia and gonorrhea were diagnosed with nucleic acid amplification of vaginal swabs using Cobas Amplicor® (Roche, Basel, Switzerland).

Blood samples testing positive for HIV were subsequently subjected to the BED-CEIA (Calypte, Portland, OR). Specimens with a normalized optical density (ODn) > 1.2 were categorized as having long-term sero-conversion, while those with an ODn ≤1.2 were subjected to confirmatory tests in triplicate, with the mean value being used as the final result. Specimens with an ODn > 0.8 were categorized as long-term sero-conversions, and those with an ODn ≤ 0.8 were categorized as recent sero-conversions. Cases with indeterminate results (n=36) were dropped from analysis, providing a sample of 2,917 FSWs. Due to quality issues in stored specimens, only 229 out of 238 specimens testing positive for HIV were tested with BED-CEIA. Incidence rates were calculated using the McDougal formula (Killian *et al*, 2006) with and without adjustment in light of recent debate regarding validity of the proposed adjustment (Brookmeyer, 2009; McDougal, 2009).

All protocols were approved by the Ethics Committee of the Indonesian Center for Biomedical and Pharmaceutical Research, Ministry of Health, and the Family Health International Protection of Human Subjects Committee.

Behavioral data were double-entered using CSPro 2.6.007 (US Census Bureau, Washington, DC) and laboratory data were entered into Microsoft Excel. Data were analyzed using Stata 9.0 (Stata Corporation, College Station, TX) and were unweighted due to incompleteness of cluster information. Demographics and sexual risk behaviors were compared across samples of FSWs; categorical variables were compared using the Wald test, means were compared with the Student's t-test, and medians were tested using the Wilcoxon rank-sum test. Differences between incidence rates were assessed using

Table 1
Percent distribution of sex workers by risk-taking and health-seeking behaviors and type of sex worker.

Characteristic	All FSW n=2,917	DFSW n=1,692	IFSW n=1,225	p-value [a]
Age				
Mean	27.9	27.1	28.4	<0.001
Median	27	27	26	<0.001
Duration of selling sex (%)				
≤ 1 year	54.3	53.2	55.7	0.400
>2 and ≤ 5 years	39.6	40.4	38.5	
> 5 years	6.1	6.4	5.7	
No. clients in past week				
Mean	8.1	10.9	4.3	<0.001
Median	5	7	3	<0.001
Always used condom with clients in past week (%)	30.2	27.1	34.8	<0.001
Condom use with boyfriend in past month (%)				
Consistent use of condoms	4.8	4.8	4.8	0.961
Inconsistent use of condoms	32.8	32.6	33.1	
No boyfriend	62.4	62.6	62.2	
Used stimulant drugs before sex in past 3 months (%)	9.4	6.4	13.6	<0.001
Report genital ulcers in past year (%)	11.8	15.5	6.6	<0.001
Completed 3 rounds of PPT in past 6 months (%)	9.2	15.0	1.7	<0.001
Treatment of last STI episode (%)				
No treatment	8.8	7.2	12.7	<0.001
Self-treatment/pharmacy	43.1	41.6	46.7	
Professional-medical	45.9	50.2	35.5	
Other	2.2	1.0	5.0	
Chlamydia PCR positive (%)	30.6	31.9	28.6	0.097
Gonorrhea PCR positive (%)	23.2	29.7	13.2	<0.001
Chlamydia PCR or gonorrhea PCR positive (%)	40.8	45.0	34.3	<0.001
Active syphilis (%)	6.1	8.2	3.0	<0.001
Tested for HIV in past year (%)	18.3	22.2	12.9	<0.001

[a] p-value pertains to difference between direct and indirect FSW

rate ratios and chi-square tests. Tests were double sided and a p-value < 0.05 was considered significant.

RESULTS

FSWs had an average age of 27.9 years and had worked as FSW for an average of 1.7 years (median 12 months) (Table 1). Fifty-four percent had worked as FSW for less than one year. Most FSW had been married, but only 8% were currently married. About 30% had given birth to a child. The average client load during the past week was 8.1 clients. Consistent condom use with clients during the past week was 30%. Forty-six percent of FSWs had their last episode of STI symptoms profession-

Table 2
HIV Incidence among female sex workers, by selected characteristics.

Characteristics	Tested for HIV (*n*)	HIV+ (*n*)	HIV prevalence (%)	BED tested (*n*)	Recent infections (*n*)	Uncorrected estimate			McDougal correction	
						Incidence[a]	95% CI	Rate ratio *p*-value	Incidence[a]	95% CI
All FSW	2,917	238	8.2	229	45	4.1	2.9-5.3	NA	3.1	2.1-4.1
Type of sex worker										
Direct	1,692	182	10.8	175	37	5.9	4.0-7.8	<0.001	4.6	2.9-6.3
Indirect	1,225	56	4.6	54	8	1.7	0.5-2.8		1.1	0.2-2.0
Age group										
<20 years	203	14	6.9	13	3	4.0	0.0-8.5	NA	3.2	0.0-7.2
20-29 years	1,656	160	9.7	153	28	4.6	2.9-6.3		3.4	1.9-4.8
≥30 years	1,058	64	6.1	63	14	3.4	1.6-5.1		2.7	1.1-4.3
City										
Bandung (West Java)	494	37	7.5	36	5	2.6	0.3-4.9	NA	1.7	0.0-3.5
Banyuwangi (East Java)	494	37	2.8	36	0	0.0	NA		NA	NA
Batam (Riau Islands)	479	51	10.7	50	13	7.2	3.3-11.1		6.1	2.5-9.6
Denpasar (Bali)	248	35	14.1	34	9	10.0	3.5-16.6		8.5	2.4-14.5
Jakarta	495	39	7.9	36	8	4.4	1.4 -7.5		3.5	0.8-6.2
Jayapura (Papua)	462	38	8.2	36	5	2.9	0.4 --5.5		1.8	0.0-3.9
Surabaya (East Java)	495	31	6.3	30	5	2.6	0.3-4.9		1.9	0.0-3.8
Duration of selling sex										
≤1 year	1,467	111	7.6	105	24	4.4	2.6-6.1	NA	3.5	1.9-5.0
>1 and ≤4 years	1,071	101	9.4	98	17	4.2	1.3-4.7		3.0	1.3-4.7
≥ 5 years	166	12	7.2	12	4	6.0	0.1-12.0		5.4	0.0 11.0
Completed 3 rounds PPT[b] in past six months										
Yes	315	28	8.9	27	1	0.9	0.0-2.5	0.045	NA	
No	2,580	210	8.1	202	44	4.5	3.2-5.8		3.6	2.4-4.7
Report genital ulcers in past year										
Yes	339	52	15.3	50	15	12.5	6.2-18.8	<0.001	10.8	4.9-16.7
No	2,547	184	7.2	177	29	3.0	1.9-4.1		2.1	1.2-3.0
Active syphilis										
Yes	169	22	13.0	22	7	11.0	2.8-19.1	0.017	9.7	2.1-17.4
No	2,616	209	8.0	200	37	3.8	2.5-5.0		2.8	1.7-3.8

[a] Per 100 person-years; [b] Periodic presumptive treatment; NA, Not available

ally treated, 9% had received three rounds of periodic presumptive treatment (PPT) for gonorrhea and chlamydia (cefixime 400 mg and azithromycin 1 g single-dose) during the past 6 months, and 18% had been tested for HIV during the past year. Twelve percent of FSWs reported having had genital ulcers during the past year, 23% tested positive for gonorrhea, 41% for either gonorrhea or chlamydia, and 6% for active syphilis.

Compared to indirect FSWs, direct FSWs were slightly younger, had more clients during the past week (mean = 10.9 *vs* 4.3), were more likely to have received professional treatment for STIs, received PPT during the past six months, been tested for HIV during the past year, reported genital ulcers during the past year, and tested positive for gonorrhea or active syphilis (all $p<0.001$). Indirect FSWs reported more consistent condom use with clients during the past week than direct FSWs ($p<0.001$).

Table 2 presents the estimates of HIV incidence for FSWs classified by selected characteristics. The overall HIV prevalence was 8.2%, with the prevalence among direct FSWs being more than twice that of indirect FSWs. The overall HIV incidence was estimated at 4.1 per 100 person years (PY) and 3.1 per 100 PY after correction. Estimated HIV incidence was more than three times higher among direct FSWs than indirect FSWs ($p<0.001$), four times higher among those reporting genital ulcers during the past year ($p<0.001$), and about three times higher among those with active syphilis ($p=0.017$). The estimated HIV incidence among those who had completed three rounds of PPT for chlamydia and gonorrhea during the past six months was five times lower ($p=0.045$). The incidence of HIV in Denpasar (Bali) and Batam (Riau Island) were 7.8 per 100 PY compared with 2.5 per 100 PY in other cities ($p<0.001$).

DISCUSSION

The BED-CEIA provides much needed information regarding HIV incidence, but has fallen into disrepute due to documented instances of overestimating HIV incidence, primarily in general population epidemics. However, several factors suggest overestimation of HIV incidence among Indonesian FSW, if present, is not likely to be substantial in magnitude. First, as general population HIV prevalence in Indonesia is low (0.2%) (Republic of Indonesia National AIDS Commission, 2008), few FSWs are likely to have been infected prior to beginning work as a FSW. Second, FSWs in Indonesia tend to have fairly short careers in sex work (median 12 months; only 6% working > 5 years), thus limiting the number of long-duration HIV infections found among FSWs at any given point in time. Finally, incidence estimates are unlikely to have been seriously distorted by antiretroviral treatment (ART) as it is estimated that only about 10% of persons eligible for ART in Indonesia are receiving treatment (Republic of Indonesia National AIDS Commission, 2008).

The findings are also internally consistent with regard to known risk factors and behavior. The observation of higher HIV incidence among direct FSWs is consistent with higher client loads and lower consistent condom use in comparison with indirect FSWs. Higher incidence rates among FSWs experiencing genital ulcers during the past year and among active syphilis cases are consistent with existing scientific knowledge concerning these factors. The lower incidence

recorded among FSWs who received PPT during the past six months is unlikely to be due to selection bias because the PPT pilot program covered a very high proportion of direct FSWs (80-100%) in the four participating cities. The higher incidence observed in Denpasar and Batam makes sense in light of relatively higher average client loads among FSWs in those cities and greater exposure to non-Indonesian clients, a higher proportion of whom may be uncircumcised in comparison with primarily Muslim Indonesian clients of FSWs in other cities.

Finally, the estimated incidence of 4.1 per 100 PY for all FSWs is consistent with the 4.7 per 100 PY estimate of the Indonesian Ministry of Health in a recent application of the Asian Epidemic Model (AEM) (Brown and Peerapatanapokin, 2004; Republic of Indonesia Ministry of Health, 2008). This is noteworthy since the tendency for the BED-CEIA to overestimate incidence *vis-à-vis* model-based estimates as well as cohort studies was an important consideration in the UNAIDS/WHO Working Group's negative recommendation concerning use of the BED-CEIA (Kartikeyan *et al*, 2007).

Information regarding HIV incidence is crucial for understanding the dynamics of HIV epidemics and targeting interventions. Although further methodological development in the measurement of HIV incidence is clearly needed, it may be that the major limitations of the BED-CEIA are less relevant, or at least not major factors, for key sub-populations in concentrated epidemics. The across-the-board dismissal of the BED-CEIA unnecessarily deprives the public health community of "usable" information for improving targeted responses to HIV in concentrated epidemics in Asia and elsewhere.

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