# Multiperson Use of Syringes Among Injection Drug Users in a Needle Exchange Program

## A Gene-Based Molecular Epidemiologic Analysis

*Sadeep Shrestha, PhD,*†‡ Michael W. Smith, PhD,*† Karl W. Broman, PhD,§ Homayoon Farzadegan, PhD,‡ David Vlahov, PhD,‖ and Steffanie A. Strathdee, PhD‡¶*

**Summary:** Syringe-sharing behaviors among injection drug users (IDUs) are typically based on self-reports and subject to socially desirable responding. We used 3 short tandem repeat (STR) genetic biomarkers to detect sharing in 2512 syringes exchanged by 315 IDUs in the Baltimore needle exchange program (NEP; 738 person-visits). Demographic characteristics as well as direct and indirect needle-sharing behaviors corresponding to the closest AIDS Link to Intravenous Experience (ALIVE) study visits were examined for association with multiperson use (MPU) of syringes. Overall, 56% of the syringes exchanged at the Baltimore NEP had evidence of MPU. Less MPU of syringes (48% vs. 71%; $P < 0.0001$) was seen with more rapid syringe turnaround (<3 days). IDUs always exchanging their own syringes ("primary" syringes) were less likely to return syringes with evidence of MPU (52%) than those who exchanged syringes for others ("secondary" syringes; 64%; $P = 0.0001$) and those exchanging primary and secondary syringes (58%; $P = 0.004$). In a multivariate analysis restricted to primary exchangers, MPU of syringes was associated with sharing cotton (adjusted odds ratio [AOR] = 2.06, 95% confidence interval [CI]: 1.30 to 3.28), lending syringes (AOR = 1.70, 95% CI: 1.24 to 2.34), and injecting less than daily (AOR = 0.64, 95% CI: 0.43 to 0.95). These findings support additional public health interventions such as expanded syringe access to prevent HIV and other blood-borne infections. Testing of STRs represents a promising approach to examining and accessing complex behavioral data, including syringe sharing.

**Key Words:** genetic biomarkers, needle exchange program, needle circulation theory, syringe sharing, syringe-sharing risk factors

(*J Acquir Immune Defic Syndr* 2006;43:335–343)

Syringe sharing has been identified as a major risk factor for the acquisition of blood-borne pathogens among injection drug users (IDUs).[1–5] "Direct" sharing takes place when an individual passes on his or her used needle and/or syringes to another, exposing that second person to residual traces of the first person's biologic material.[6,7] Likewise "indirect" sharing of syringes by means of "backloading" and "frontloading"[6,8,9] or sharing of injection paraphernalia such as cookers, cotton filters, and rinsing water[10,11] can expose users to infectious agents, including HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV).[9,12–16] Several studies have reported that the prevalence of injection paraphernalia sharing is 2 to 3 times greater than that of direct needle sharing.[13,17,18]

Needle exchange programs (NEPs) have been deployed as a harm reduction strategy to prevent the acquisition of blood-borne infections among IDUs.[19,20] A major objective of NEPs is to decrease the circulation of potentially contaminated multiperson-use (MPU) syringes in the community, thereby lowering the incidence of blood-borne infections.[21,22] Several studies have shown that NEPs are associated with decreased needle sharing,[18,23–26] reductions in HIV prevalence,[27,28] and reductions in HIV incidence.[29,30]

Almost all NEP evaluation studies have relied on self-reports of needle sharing among attendees.[31] Based on self-reports, needle-sharing behavior is high among IDUs attending NEPs at baseline[18,23] but significantly declines over time among HIV-seronegative[2,18,23,32,33] and HIV-seropositive NEP attendees.[24,34] Self-reported behaviors of IDUs are generally valid;[35] however, they may be prone to socially desirable responding.[36,37] Recent studies using audio computer-assisted self-interview (ACASI) indicate that IDUs tend to underreport sensitive behaviors such as needle sharing during face-to-face interviews.[38,39]

Although self-reported data, if interpreted cautiously, can be used to study IDU behaviors, molecular assessment of DNA from used syringes promises to provide another level of certainty about risk behaviors. Obtaining valid biologic markers for MPU syringe analysis minimizes the potential biases and limitations of self-reports. Short tandem repeats

Received for publication January 27, 2006; accepted May 25, 2006.

From the *Laboratory of Genomic Diversity, National Cancer Institute (NCI)-Frederick, Frederick, MD; †Basic Research Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; ‡Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; §Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; ‖Centers for Urban Epidemiologic Studies, New York Academy of Medicine, New York, NY; and ¶Division of International Health and Cross Cultural Medicine, University of California, San Diego, School of Medicine, La Jolla, CA.

S. Shrestha's current address is Department of Epidemiology and International Health, School of Public Health, University of Alabama-Birmingham, AL 35294.

Funded in part with federal funds from the National Cancer Institute (NCI), National Institutes of Health (NIH), under contract N01-CO-12400 and the National Institute on Drug Abuse (grants DA09225, DA12568, and DA04334). Supported in part by the Intramural Research Program of the NIH, NCI, Center for Cancer Research.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Reprints: Michael W. Smith, PhD, Laboratory of Genomic Diversity, SAIC-Frederick, Inc., NCI-Frederick, Building 560, Room 21-74, Frederick, MD 21702 (e-mail: smithm@ncifcrf.gov).



**Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.**

(STRs) are a type of genetic marker for which theoretic and laboratory approaches have been previously developed for syringe residue analysis.[40,41] Briefly, STRs are tandemly repeated simple DNA sequence motifs that are 2 to 7 bases in length.[42–44] STRs are characterized by Mendelian inheritance and are widely dispersed in the genome of all racial, ethnic, and geographically defined populations,[45–47] making them useful in forensic identification applications and genetic epidemiology. Allelic polymorphisms originate as mutations caused by slipped-strand mispairing during DNA replication.[48,49] The variability results from the gain or loss of repeat units, with mutation rates typically ranging from $10^{-3}$ to $10^{-5}$ events per gamete per generation.[50,51] Detecting 3 or more alleles at an STR in a single biologic sample provides evidence of biologic mixture from at least 2 individuals.

Improved documentation of syringe-sharing behaviors among IDUs could provide insights to preventing and controlling epidemics of HIV and other blood-borne infections. The purpose of this study was to study the extent of MPU of syringes returned to the Baltimore NEP based on genetic biomarkers (pentanucleotide STRs)[41] and to examine the association between MPU of syringes and self-reports of needle-sharing behavior in a retrospective study of NEP attendees.

## METHODS

### Study Samples

The study design and methods for the AIDS Link to Intravenous Experience (ALIVE) study have been previously described.[52,53] Briefly, the ALIVE study comprises a cohort of IDUs in Baltimore who were prospectively followed up to study the natural history and risk factors of HIV/AIDS.[54] At each study visit, participants completed a detailed interviewer-administered questionnaire (IAQ) that collected information on demographics; personal health; sexual practices; and injection behaviors, including sharing of syringes and other paraphernalia (ie, cookers, cotton).

The Baltimore NEP was authorized by state legislation on August 12, 1994 and was initiated with the funding from the Baltimore City Health Department. During the study period, the NEP provided one of the only legal sources of sterile needles in Maryland. The program was operated through mobile vans and the pharmacy-based exchange sites. At initiation, trained staff conducted a brief 21-item registration survey. At baseline, participants were provided with 2 sterile syringes, cookers, sterile cotton, condoms, and HIV information brochures, and in their subsequent visits, they exchanged the used syringe for a sterile syringe in unlimited numbers on a 1:1 basis. On request, HIV testing with pre- and post-test counseling was available, along with tuberculosis skin testing and referrals to subsidized drug treatment.

### Syringe Samples

Bar-coded syringes were dispensed between September 1994 and February 1997, whereby 746,029 syringes were exchanged by more than 5369 IDUs, and 197,216 of these were returned. All bar-coded syringes were nonnominally linked to each subject's unique identifier, enabling the identification of the individual(s) who acquired or returned any particular syringe from the Baltimore NEP, and also linked to the ALIVE study cohort, although preserving confidentiality. Between January 1995 and February 1996, a random number of 13,399 syringes exchanged by 893 IDUs were retained and rinsed with washing buffer, and their residues were stored at $-70°C$, as described more fully elsewhere in this report. In the present study, only the syringes with ALIVE study questionnaire data on the exchanger corresponding to the 6-month period of the visit to the Baltimore NEP were used. We excluded 4 individuals who exchanged more than 30 syringes per visit, because previous studies have shown that high-volume exchangers are those who sell syringes to others.[55] In this study, we examined a total of 2512 syringes exchanged by 315 ALIVE study participants during 728 NEP person-visits (Fig. 1).

### Syringe Exchanger Type

Barcode data linking individuals to each syringe returned per visit were used to create a matrix characterizing the type of syringe exchanger. During each visit, some IDUs returned only the syringes that they acquired ("primary" syringes), others exchanged only the syringes that were acquired by others ("secondary" syringes), and some exchanged primary and secondary syringes.

### Laboratory Methods

Three STRs [W (D1S71752), X (D4S18742), and Z (D2S17842)] were used as biomarkers for validating self-reports of syringe sharing. The characteristics of the STRs (heterozygosity [H], an extent of allelic polymorphism; the probability of mixture discrimination [$P_{MD}$], an estimate of the chance of detecting a third allele in a biologically mixed sample of the STRs (Table 1); and validity [sensitivity and specificity]) have been described previously.[41,56]

A stepwise laboratory procedure, including syringe washing, DNA extraction, polymerase chain reaction (PCR) amplification reaction with STRs, and fragment separation and sizing, was performed to differentiate between single person use (SPU) or MPU of syringes as previously described.[41] Briefly, washing buffer consisted of 10 mM of Tris hydrochloric acid (HCl), pH 8.3, 50 mM of potassium chloride (KCl), 2.5 mM of magnesium chloride ($MgCl_2$), Tween 20, and NP40. Fifty microliters of the buffer was used to dissolve the residues in the syringes thoroughly. DNA was extracted using a 96-format DNAamp blood kit (Qiagen, Hilden, Germany). A PCR assay was carried out separately for each STR genetic marker using the primers and conditions previously reported.[56]

PCR fragments were separated by size using capillary electrophoresis. Because the fluorescent PCR products have upper detection thresholds in the Applied Biosystems 3100 genetic analyzer system, we used the Genescan and Genotyper programs (Applied Biosystems, Foster City, CA) to limit the peak heights of alleles to a maximum of 8000 relative fluorescent units (rfu) and reran the sample at lower concentrations (1/10 and 1/100) searching for suitable signal intensities. Two laboratory personnel visually inspected each electropherogram plot for all peak heights <1000 rfu and determined the minimum threshold height to be the sum of the 2 highest STR



Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

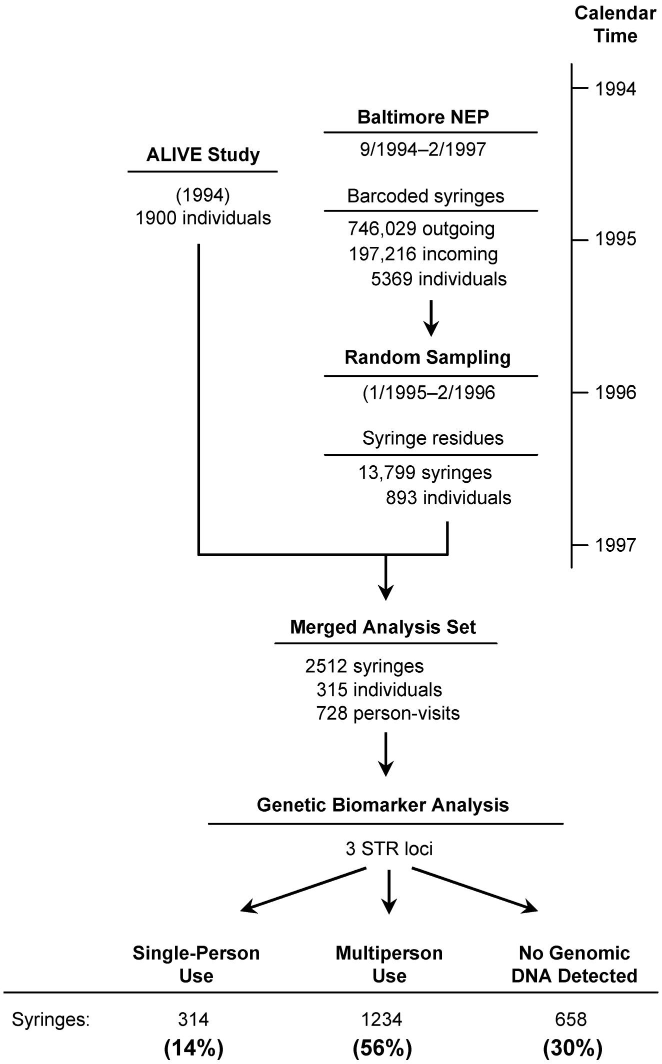


**FIGURE 1.** Study design and results of assessing syringe sharing. Genetic biomarker analyses of 3 STRs was performed on an overlapping set of 2512 syringes from 315 individuals for comparison to ALIVE study self-reports on risk behaviors. Syringes with 1 or 2 alleles observed in at least 2 loci examined were classified as SPU syringes, and those with 3 or more alleles at any loci were classified as MPU syringes.

alleles [500 rfu for W (D1S71752) and Z (D2S17842) and 850 rfu for X (D4S18742)] based on data quality.

## Mixture Determination Algorithm

The quantitative algorithm based on STR peak height for determining true alleles versus the artifactual products or typical stutter has been described previously.[56] Briefly, the algorithm accounted for all possible stutters resulting from all the STR peaks and created a matrix of the expected stutter reference height at each allelic position. A true allele was differentiated if the observed peak at any allelic position was greater than the expected reference. Other misclassifications resulting from sensitive biomaterial amplification detection have been previously described, and our stringent algorithm for allele calling aims to maximize specificity in our analyses.[41,56] Application of the algorithm in laboratory simulated samples yielded sensitivities of 77.5%, 82.7%, and 58% while maintaining the high specificities of 100%, 97.4%, and 100% for the W, X, and Z STRs individually as well as a collective sensitivity of 91.4% and a collective specificity of 97.4%.[56]

## Multiperson Use, Single Person Use, and No Detectable Genomic DNA

Based on the genomic DNA contents, syringes were categorized as MPU, SPU, or "no detectable genomic DNA" (NDGD) as follows. The presence of 3 or more alleles was used to categorize syringes as MPU, and the presence of 1 or 2 alleles was used to categorize syringes as SPU. Thus, if any single STR resulted in evidence of MPU, the syringe was considered to be shared by at least 2 individuals. Conversely, if 2 or more STRs independently indicated SPU (or had missing data on the third STR), the syringe was considered to have been used by only 1 individual. NDGD indicated that the syringe had never been used (which is unlikely) or had been sterilized and washed properly before being returned to the NEP, leaving no traceable biologic materials (we are able to detect as little as 0.1 ng of DNA and mixtures with ratios as low as 95:5[41]). SPU syringes combined with NDGD in all STRs were grouped as non-MPU "safe" syringes in the analyses. These categorizations were used considering that from the public health perspective, non-MPU syringes indicate that the syringes were safely used, whereas MPU syringes indicate that the syringes were not safely used and could therefore transmit blood-borne pathogens.

## Statistical Analysis

Each syringe was analyzed first to determine the genomic content as described in the Methods section. Syringes exchanged at each NEP person-visit by the participants were then used as the unit of analysis to estimate the proportion of MPU syringes returned to the program by each individual. The Cochran-Armitage trend test was performed to examine the trend in the proportion of MPU syringes as circulation time increased (ie, 1–3, 4–10, 11–20, 21–40, and more than 40 days). MPU syringes were also examined by the syringe exchanger type.

Further, among the primary exchangers, the level of MPU of syringes was examined to assess the associations with their demographic characteristics (age, gender, race, and HIV status) and self-reported injecting behaviors (lending syringes, borrowing syringes, sharing syringes, sharing cookers, sharing cotton, and attending shooting galleries). We used self-reported data from the ALIVE study visit that was closest in time to the Baltimore NEP visit when the syringe(s) were exchanged. The analysis was performed first with MPU versus SPU syringes only and then with MPU versus non-MPU syringes to assess safe versus "unsafe" use of syringes.

Using these demographic and self-reported variables, we identified predictors of needle sharing using a logistic regression model, with the response variable being the binomial proportion of MPU syringes for each NEP person-visit. The analysis was performed with logit link in SAS



Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

**TABLE 1.** Characteristics of Genetic Biomarkers and Determination of MPU, SPU, and NDGD

| STR | Repeat Sequence | H* | $P_{MD}$* | MPU† n (%) | SPU‡ n (%) | NDGD§ n (%) |
|---|---|---|---|---|---|---|
| W (D1S71752) | $ttgca_{9–23}$ | 0.79–0.82 | 0.74–0.80 | 938 (43) | 502 (23) | 766 (35) |
| X (D4S18742) | $cgata_{12–23}$ | 0.77–0.81 | 0.71–0.79 | 529 (24) | 549 (25) | 1128 (51) |
| Z ( D2S17842) | $cagca_{14–23}$ | 0.68–0.82 | 0.57–0.81 | 1234 (56) | 314 (14) | 658 (30) |

*H and $P_{MD}$ value range in African-American and European-American populations.[56]
†MPU ≥3 STR alleles.
‡SPU ≤2 STR alleles.
§NDGD when no amplified PCR product was detected.
All our positive control samples were amplified, and the alleles were correctly matched. None of our blank samples were amplified by PCR. Because the amount of DNA was not easy to titrate, a standard 10 μL of DNA from the extraction solution was used for PCR amplification in a total volume of 15 μL. The STR genetic markers examined have been previously described in detail relative to PCR conditions and amplification primers.[56]
"Unsafe" syringes included MPU syringes only and the "safe" syringes included the SPU and NDGD syringes. Because there is no genomic detected (with all 3 STRs), it is assumed that there are no biologic materials, including the infectious agents; thus, these syringes were not used previously and/or they were disinfected properly (ie, washed or bleached);[59] thus, reusing the syringe would be a safe practice from the infection transmission point.

(version 9.0) using the Proc Genmod function. Generalized estimating equations (GEEs)[57] were used to account for correlation between multiple visits for the same individuals using an exchangeable correlation structure.[58] Models also included an adjustment for the length of follow-up in the ALIVE study, because individuals who had been enrolled for a longer time were expected to be more prone to socially desirable responding. Variables that were significant at the 10% level in univariate analyses were also included in the multivariate analyses. All plausible 2-way interactions were explored. In each analysis, events that were missing relevant information because of nonresponse were excluded.

## RESULTS

Individuals who acquired the syringes (n = 211) were not significantly different from those who returned them (n = 176) in terms of age (34.9 vs. 34.8 years), gender (73.9 vs. 74.4% male), race (96.1 vs. 95.5% African American), and HIV serostatus (47.8 vs. 49.9% HIV-positive). There was a median of 2 NEP visits per person (interquartile range [IQR]: 1–3) and 3 syringes exchanged per visit (IQR: 2–5).

Residues obtained from 1234 syringes (56% of the syringes tested) were found to have evidence of MPU, 314 (14%) had evidence of SPU, and 658 (30%) had NDGD (Table 2). Overall, significant increases in proportions of MPU syringes were observed the longer that syringes had been in circulation (Fig. 2; trend test, $P = 0.019$). Syringes with the shortest circulation times (1–3 days) were shared less frequently than those circulating for longer periods (48% vs. 71%, $\chi^2 = 114.26$, 1 degree of freedom; $P < 0.001$).

The prevalence of MPU was approximately 80% overall when only SPU and MPU syringes were used for the analyses (excluding those without detectable DNA). Given such high prevalence, none of the variables (syringe type, demographics, or self-reported risk behaviors) were associated with exchanging MPU syringes (analysis not shown). Further examination with exchanging unsafe syringes (ie, those indicating MPU) compared with safe syringes (ie, those indicating SPU or NDGD) led to a number of significant associations. Compared with individuals exchanging only primary syringes, return of MPU syringes versus return of SPU syringes was more likely for those exchanging primary and secondary syringes (64% vs. 58%, odds ratio [OR] = 1.79, 95% confidence interval [CI]: 1.33 to 2.40) and for those exchanging only secondary syringes (64% vs. 52%, OR = 2.07, 95% CI: 1.25 to 3.44).

In the analyses restricted to the primary exchangers, there were several self-reported risk behaviors associated with unsafe use of syringes. Individuals who said they lent syringes (61% vs. 50%, OR = 1.16, 95% CI: 1.02 to 1.52), shared syringes (60% vs. 50%, OR = 1.19, 95% CI: 0.96 to 1.92), or shared cotton (63% vs. 50%, OR = 1.56, 95% CI: 1.15 to 2.15) were more likely to return syringes with evidence of MPU. Those who reported injecting less than once a day were less likely than those injecting more than daily to share syringes (47% vs. 55%, OR = 0.88, 95% CI: 0.69 to 0.93). Altogether, in the final multivariate model (Table 3), exchanging MPU syringes was independently associated with self-report of sharing cotton (adjusted odds ratio [AOR] = 2.06, 95% CI: 1.30 to 3.28), lending syringes (AOR = 1.70, 95% CI: 1.24 to 2.34), and injecting less than daily (AOR = 0.64, 95% CI: 0.43 to 0.95).

Interestingly, the HIV serostatus of the subjects who acquired and returned syringes was not associated with MPU of syringes. To explore syringe use behaviors among high-risk and low-risk individuals, we also examined the secondary syringes exchanged by HIV-concordant (both individuals acquiring and returning the syringes were HIV-seropositive) and HIV-discordant pairs (where only 1 individual was HIV-seropositive), but no significant differences were observed (analysis not shown). Likewise, there was no difference in DNA detection between syringes exchanged by individuals who reported that they bleached syringes versus those who did not (analysis not shown).

## DISCUSSION

The use of genetic biomarkers in conducting genomic analysis of syringe residues provides a promising methodology for differentiating MPU versus SPU of syringes. A unique feature of our study was the ability to link the syringes to the individuals who acquired or returned them at an NEP and to the self-reported behaviors from an ongoing cohort study. The present study was conducted with syringes from the Baltimore NEP in 1995 through 1996, and thus may not reflect the extent of MPU of syringes among current NEP attendees. It lays



Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

**TABLE 2.** Univariate Analysis of MPU and Non-MPU of Syringes Based on Demographics and Self-Reported Behaviors

| Variables | No. Syringes (Person-Visit)* | Proportion of MPU Syringes | OR (95% CI)† | *P* |
|---|---|---|---|---|
| Syringe type | | | | |
| Syringe exchange type | | | | |
| Secondary only | 238 (74) | 0.64 | 2.07 (1.25 to 3.44) | 0.004 |
| Primary and | 549 (173) | 0.58 | 1.79 (1.33 to 2.40) | <0.0001 |
| Secondary | 1419 (483) | 0.52 | 1.00 | |
| Primary only | | | | |
| Demographics‡ | | | | |
| Age (y) | | | | |
| >35 | 1207 (388) | 0.52 | 1.22 (0.84 to 1.76) | 0.29 |
| ≤35 | 212 (95) | 0.54 | 1.00 | |
| Gender | | | | |
| Male | 1060 (364) | 0.52 | 0.82 (0.63 to 1.07) | 0.09 |
| Female | 316 (115) | 0.58 | 1.00 | |
| Race | | | | |
| Other | 32 (18) | 0.68 | 1.48 (0.72 to 3.33) | 0.28 |
| African American | 1387 (465) | 0.52 | 1.00 | |
| HIV | | | | |
| Positive | 630 (226) | 0.54 | 1.05 (0.84 to 1.31) | 0.82 |
| Negative | 789 (257) | 0.52 | 1.00 | |
| Risk behavior self-reports‡ | | | | |
| Lend syringes | | | | |
| Yes | 305 (82) | 0.61 | 1.16 (1.02 to 1.52) | 0.04 |
| No | 1113 (397) | 0.50 | 1.00 | |
| Borrow syringes | | | | |
| Yes | 184 (53) | 0.61 | 1.39 (0.93 to 2.08) | 0.26 |
| No | 1234 (426) | 0.53 | 1.00 | |
| Share syringes | | | | |
| Yes | 229 (71) | 0.60 | 1.19 (0.96 to 1.92) | 0.09 |
| No | 1179 (404) | 0.50 | 1.00 | |
| Share cookers | | | | |
| Yes | 489 (156) | 0.54 | 0.88 (0.70 to 1.12) | 0.61 |
| No | 887 (308) | 0.52 | 1.00 | |
| Share cotton | | | | |
| Yes | 237 (80) | 0.63 | 1.56 (1.15 to 2.15) | 0.03 |
| No | 1138 (384) | 0.50 | 1.00 | |
| Visit a shooting gallery | | | | |
| Yes | 26 (7) | 0.57 | 1.07 (0.052 to 2.11) | 0.74 |
| No | 1350 (457) | 0.52 | 1.00 | |
| Injection frequency | | | | |
| <Daily | 1027 (359) | 0.47 | 0.88 (0.69 to 0.93) | 0.009 |
| >Daily | 396 (124) | 0.55 | 1.00 | |
| Bleach use | | | | |
| Yes | 631 (181) | 0.55 | 1.04 (0.83 to 1.30) | 0.32 |
| No | 745 (283) | 0.51 | 1.00 | |

*Total number of syringes returned at the total person-visits by exchanger.
†OR and 95% CI (adjusted for number of ALIVE study visits) using GEE with logistic regression.
‡Restricted to primary exchangers with self-reported risk behavior information.

a foundation for applying genetic biomarkers to study behavioral characteristics such as needle sharing, however.

Using a genomic analysis of 3 STRs, more than half (56%) of the Baltimore NEP syringes we studied had evidence of MPU. The prevalence of MPU of syringes we observed is higher than that of previous reports during the same period but could be attributable to the enrollment of high-risk individuals in the Baltimore NEP. For example, at baseline in the ALIVE study, which predated the introduction of NEPs, HIV and HCV prevalences were 25% and 88%, respectively.[52] Based on our



Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

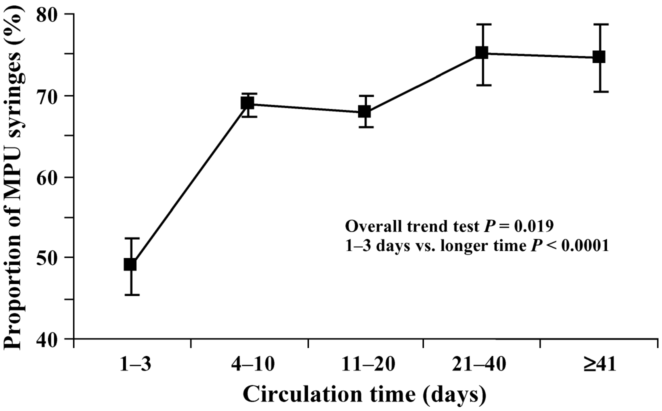


**FIGURE 2.** Proportion of MPU of syringes by duration of circulation. The syringes were divided into 5 categories based on their circulation time (1–3 days, 4–10 days, 11–20 days, 21–40 days, and ≥41 days), and the proportion of MPU syringes was calculated (48%, 68%, 67%, 75%, and 74%, respectively). Sharing increases with time (trend test, $P = 0.019$) and especially after the first 3 days (48% vs. 71%, $\chi^2 = 114.26$, 1 degree of freedom; $P < 0.0001$). The proportion of shared syringes plateaus after day 3 at approximately 75%, suggesting the existence of a reservoir of SPU syringes.

analysis, 30% of the syringes had no evidence of genomic DNA. These syringes had never been used before or had been washed thoroughly with syringe cleaners (eg, bleach, detergent, soap), or they were left in extreme conditions (eg, heat) before specimen collection, resulting in DNA degradation. Samples were stored frozen after phosphate-buffered saline washes were performed until they were thawed for DNA extraction. DNA from archived material, as in our case, has been routinely analyzed in forensic cases; thus, degradation attributable to syringe residue storage is unlikely, although unknown conditions affecting the syringes could have affected the genetic tests by degrading the DNA. We did not test for trace amounts of perchlorate in the syringe residues; however, admittance of bleach use made no difference in DNA detection. We do not know whether the index person bleached the syringe before reusing it, whether the last user bleached the syringe before returning it to the NEP, or if the syringe was cleaned in any way at all. Bleach disinfects and inactivates bacteria and viruses within a few minutes. The effectiveness of a bleach depends on several factors, however, including concentration and time of exposure.[59] Several studies have suggested that IDUs do not clean their syringes effectively with bleach.[60,61] We did not find any difference in MPU of syringes between those who admitted bleaching and those who did not, but we adjusted for this in all our analyses. If human biologic materials could not be detected in the residues, we inferred that most infectious microbes and viruses could not have survived.

**TABLE 3.** Independent Predictors of Exchanging MPU Syringes

| Variable | AOR (95% CI)* | *P* |
|---|---|---|
| Share cotton | | |
| Yes | 2.06 (1.30 to 3.28) | 0.0022 |
| No | 1.00 | |
| Lend syringes | | |
| Yes | 1.70 (1.24 to 2.34) | 0.0011 |
| No | 1.00 | |
| Injection frequency | | |
| <Daily | 0.64 (0.43 to 0.95) | 0.03 |
| >Daily | 1.00 | |

*AOR and 95% CI (multivariate analysis using GEE with logistic regression, adjusted for number of ALIVE study visits).

Other studies have also indicated that NEPs may attract individuals who inject more frequently and engage in high-risk activities such as sharing needles.[18,62,63] Nearly half of the participants in our study were already HIV-seropositive, and 96% were HCV-seropositive. The prevalence of HIV is much higher in our study samples than in IDUs attending the NEP (29.5%) or participating in the ALIVE study (32%) during this same period,[34,53] suggesting their involvement in high-risk behaviors even before attending the NEP. The fact that needles continued to be a commodity in Baltimore after the NEP opened attests to the fact that there remained an inadequate number of syringes exchanged in the city to meet the demand. During the period when the syringes for the present study were collected, the Baltimore NEP operated a van 4 days per week for 2 hours daily each on the east and west sides of the city. Although the hours and locations have been expanded in subsequent years, more work is needed to target the high-risk individuals who are hard to reach.

In other cities, for example, Montreal, <5% of the estimated syringe demand was being met during the escalating HIV incidence among IDUs.[64] In Vancouver, it was estimated that more than double the amount of syringes was required on an annual basis to provide a sterile syringe for every injection, despite the effort to distribute more than 2 million syringes per year since 1996.[65] Likewise, low syringe coverage in the late 1990s was the main reason attributed to an HIV outbreak in Kathmandu, Nepal.[66] The fact that a significant difference in MPU of syringes was observed among those that were circulating for 1 to 3 days compared with the longer period supports the idea that the turnover rate of exchanged syringes needs to be increased to reduce the likelihood of a syringe being shared. According to Kaplan and Heimer's theory[67] of syringe circulation, the longer the syringe is out, the more likely it is that it is contaminated with HIV infection. Thus, provision of adequate syringes decreases the turnaround time during which potentially contaminated syringes may infect others. We found that injecting less than once daily was associated with decreased MPU of syringes. Increased injection frequency has been associated with HIV seroconversion in several studies.[53,68]

We also found that primary exchangers were less likely to share syringes than those who returned syringes acquired by others. This is not surprising, because a previous study has indicated that exchanging one's own syringes was significantly associated with lower levels of receptive needle sharing, backloading, sharing other injection equipment, and lending used needles.[69] We assumed that the individuals used the



Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

syringes they exchanged, and this could be a major limitation of our study. Self-reports may not reflect the actual user's behaviors, which would affect the validation analyses.

Interestingly, 35% of our subjects indicated that they did not inject drugs in the past 6 months in their interview. We did not see any significant differences in MPU of syringes between those who self-reported not injecting in the past 6 months versus those who did. Their participation in the NEP suggests response errors in their interviews or that they were exchanging syringes for someone else, which is known to be quite common in Baltimore.[70] Although we excluded high-volume exchangers (4 individuals with more than 30 syringes per NEP visit), who are known to sell syringes, we did not confirm that the syringes we sampled were used by the exchangers themselves. To confirm that the person who acquired the syringe at the NEP actually used it, forensic typing and subsequent analyses could be conducted in the future to match the STR allelic pattern from stored blood of each cohort participant to the residue contents present in the syringes. Although this would minimize misclassification and strengthen the inferences that can be drawn from this analysis, caution is needed regarding the ethics of conducting DNA profiling studies. Although the practice of secondary syringe exchange may be beneficial in reaching out to more hidden IDUs who do not use an NEP, they may not be exposed to the ancillary services (eg, condoms, prevention education, treatment referrals) that the program provides.

Although, there was no absolute agreement between the self-reports and the extent of needle sharing, our data suggest that MPU of syringes is more common in those who report direct or indirect sharing. Unaccounted indirect sharing[9,13,17] could be another explanation of the high prevalence of MPU syringes despite the lower rate of self-reported injection risk behaviors in our study. In a study of IDUs in Denver, Koester et al[71] found that 72% reported indirect sharing in the last 30 days, which was twice the rate of direct needle sharing. IDUs in many cities remain unaware of indirect sharing behaviors as risk factors for the spread of blood-borne infections.[10,71,72] Another study[17] reported that only 7% of IDUs were aware that indirect sharing represented a risk of becoming infected with HIV. Vlahov et al[18] previously reported prevalence of backloading, sharing cookers, sharing cotton, and attending shooting galleries among Baltimore NEP attendees at baseline to be 11.7%, 60.5%, 45.8%, and 22.9%, respectively. In our study, one third of IDUs reported sharing cookers and one fifth reported sharing cotton, but we lacked data on other indirect sharing of syringes and injection paraphernalia such as backloading, frontloading, and sharing rinse water. In fact, we found that sharing cotton was an independent predictor of exchanging MPU syringes. Injectors draw up drug solutions into their syringes through the cotton, which is used to filter out particulate matter. In such cases, the syringe of the second user can be contaminated with the biologic material from the first user and/or from the cooker. Thus, even if subsequent individuals use a sterile syringe or rinse the used syringe thoroughly with water, reuse of cotton can contaminate the syringe. Contact with cotton is the last process during the preparation of the drug solution before injection; thus, any contamination of the syringes, cookers, or water can also contaminate the cotton. Sharing cotton has been identified as an independent risk factor for HCV infection in several studies.[15,16,73]

In contrast, forms of direct sharing other than lending syringes were not associated with MPU of syringes. IDUs may less readily engage in some forms of direct sharing, especially borrowing syringes from others (often referred to as receptive syringe sharing), but may feel comfortable admitting behaviors that are less socially unacceptable, such as lending to others (distributive syringe sharing) or indirect sharing. Also, in cases in which multiple persons engage in needle sharing, the order of syringe use is important, especially if the distributive user is infected with HIV and the receptive user is not. Although at an individual level, syringe use between seroconcordant individuals may not reflect as much risk as between serodiscordant individuals, sharing syringes generally remains a major risk factor for transmission of infectious disease at the population level.

In our study, some cohort participants were recruited as early as 1988, which is much earlier than our study period, and might have developed a close rapport with the interviewers, making them prone to socially desirable responding. We adjusted for follow-up time in our analysis to minimize the potential for this bias, however. Also, at semiannual study visits, participants are provided with risk reduction counseling, which may potentiate the tendency for underreporting risk behaviors. In addition, generalization of self-reported behaviors over a 6-month period may have confounded our results, because behaviors like needle sharing change over time. Nevertheless, given our study design, there were only a few syringe samples from each person over a relatively short follow-up period; thus, we did not have sufficient power to evaluate the change in pattern of sharing over time.

Our study has directly measured sharing of syringes through the use of genetic biomarkers for the first time. Our novel approach has important implications for future studies evaluating NEP effectiveness, understanding patterns of direct and indirect needle sharing, and potentially extending the method to unobtrusive public health surveillance of discarded street syringes in communities. Additionally, detection of DNA, RNA, or antibodies of infectious viruses such as HIV, HCV, and HBV in syringe exudates, along with sharing information, would be valuable in exploring models of transmission routes and pattern of infectious diseases among IDUs at the individual and population levels. Although there are concerns about self-reports, retrieving discarded needles using previously described street sampling methods[74] and testing them using this novel molecular method could provide estimates over time on rates of needle sharing in communities as a public health alert.

## ACKNOWLEDGMENTS

*The authors thank Dr. Stephen J. O'Brien, Dr. Marilyn Raymond, Dr. Taras Oleksyk, Dr. Terri Beaty, Dr. Ronald Brookmeyer, and Victor David for helpful insights in developing the ideas for this research. They are also grateful to Dr. Yvette Berthier-Schaad, Ann Truelove, Mahboobeh Safaeian, Kai Zhao, and Joseph Bareta for their assistance.*



**Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.**

## REFERENCES


1. Alter MJ, Hadler SC, Margolis HS, et al. The changing epidemiology of hepatitis B in the United States—need for alternative vaccination strategies. *JAMA*. 1990;263:1218–1222.
2. Hagan H, Des Jarlais DC, Friedman SR, et al. Reduced risk of hepatitis-B and hepatitis-C among injection-drug users in the Tacoma syringe exchange program. *Am J Public Health*. 1995;85:1531–1537.
3. Levine OS, Vlahov D, Brookmeyer R, et al. Differences in the incidence of hepatitis B and human immunodeficiency virus infections among injecting drug users. *J Infect Dis*. 1996;173:579–583.
4. Kral AH, Bluthenthal RN, Erringer EA, et al. Risk factors among IDUs who give injections to or receive injections from other drug users. *Addiction*. 1999;94:675–683.
5. Thomas DL, Astemborski J, Rai RM, et al. The natural history of hepatitis C virus infection—host, viral, and environmental factors. *JAMA*. 2000; 284:450–456.
6. Koester S. Following the blood: syringe reuse leads to blood-borne virus transmission among injection drug users. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;18(Suppl):S139–S140.
7. Rich JD, Dickinson BP, Carney JM, et al. Detection of HIV-1 nucleic acid and HIV-1 antibodies in needles and syringes used for non-intravenous injection. *AIDS*. 1998;12:2345–2350.
8. Vlahov D. Backloading and HIV infection among injection drug users. *Int J Drug Policy*. 1996;7:52–57.
9. Needle RH, Coyle S, Cesari H, et al. HIV risk behaviors associated with the injection process: multiperson use of drug injection equipment and paraphernalia in injection drug user networks. *Subst Use Misuse*. 1998;33: 2403–2423.
10. Koester A, Booth R, Wiebel W. The risk of HIV transmission from sharing water, drug mixing containers and cotton filters among intravenous drug users. *Int J Drug Policy*. 1990;1:28–30.
11. Grund J, Friedman SR, Stern L, et al. Syringe-mediated drug sharing among injecting drug users. Patterns social context and implications for transmission of blood-borne pathogens. *Soc Sci Med*. 1996;45:691–703.
12. Des Jarlais DC, Stimson GV, Hagan H, et al. Injection drug use and emerging blood-borne diseases. *JAMA*. 1996;276:1034.
13. McCoy CB, Metsch LR, Chitwood D, et al. Parenteral transmission of HIV among injection drug users: assessing the frequency of multiperson use of needles, syringes, cookers, cotton and water. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;18(Suppl 1):S25–S29.
14. Shapshak P, Fujimura RK, Page JB, et al. HIV-1 RNA load in needles/ syringes from shooting galleries in Miami: a preliminary laboratory report. *Drug Alcohol Depend*. 2000;58:153–157.
15. Hagan H, Thiede H, Weiss NS, et al. Sharing of drug preparation equipment as a risk factor for hepatitis C. *Am J Public Health*. 2001;91: 42–46.
16. Thorpe LE, Ouellet LJ, Hershow R, et al. Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. *Am J Epidemiol*. 2002;155:645–653.
17. Koester S, Hoffer L. Indirect sharing: additional HIV risks associated with drug injection. *AIDS Public Policy J*. 1994;9:100–105.
18. Vlahov D, Junge B, Brookmeyer R, et al. Reductions in high-risk drug use behaviors among participants in the Baltimore needle exchange program. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997;16:400–406.
19. van Ameijden EJ, van den Hoek AR, Coutinho RA. Injecting risk behavior among drug users in Amsterdam, 1986 to 1992, and its relationship to AIDS prevention programs. *Am J Public Health*. 1994;84: 275–281.
20. Strathdee SA, Hogg RS, Martindale SL, et al. Determinants of sexual risk-taking among young HIV-negative gay and bisexual men. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;19:61–66.
21. Vlahov D, Junge B. The role of needle exchange programs in HIV prevention. *Public Health Rep*. 1999;113:75–80.
22. Geyser D. Needle exchange program funding. *Harvard J Legis*. 2000;37: 265–278.
23. Bluthenthal RN, Kral AH, Gee L, et al. The effect of syringe exchange use on high-risk injection drug users: a cohort study. *AIDS*. 2000;14: 605–611.
24. Vertefeuille J, Marx M, Tun W, et al. Decline in self-reported high risk injection-related behaviors among HIV seropositive participants in Baltimore needle exchange program. *AIDS Behav*. 2000;4:381–388.
25. Ksobiech K. A meta-analysis of needle sharing, lending, and borrowing behaviors on needle exchange program attender. *AIDS Educ Prev*. 2003; 15:257–268.
26. DeSimone J. Needle exchange programs and drug infection behavior. *J Policy Anal Manage*. 2005;24:559–577.
27. Heimer R, Khoshnood K, Stephens PC, et al. Needle exchange decreases the prevalence of HIV-1 proviral DNA in returned syringes in New Haven, Connecticut. *Am J Med*. 1996;95:214–220.
28. Hurley SF, Jolley D, Kaldor J. Effectiveness of needle-exchange programmes for prevention of HIV infection. *Lancet*. 1997;349:1797–1800.
29. Gibson D, Flynn N, Perales D. Effectiveness of syringe exchange programs in reducing HIV risk behavior and HIV seroconversion among injecting drug users. *AIDS*. 2001;15:1329–1341.
30. Heimer R, Clair S, Teng W, et al. Effects of increasing syringe availability on syringe-exchange use and HIV risk: Connecticut, 1990–2001. *J Urban Health*. 2002;79:556–570.
31. Bastos FI, Strathdee SA. Evaluating effectiveness of syringe exchange programmes: current issues and future prospects. *Soc Sci Med*. 2000;51: 1771–1782.
32. Des Jarlais DC, Marmor M, Paone D, et al. HIV incidence among injecting drug users in New York City syringe-exchange programmes. *Lancet*. 1996;348:987–991.
33. Hagan H, Thiede H. Changes in risk behavior associated with participation in Seattle needle exchange program. *J Urban Health*. 2000;77:369–382.
34. Vertefeuille J, Strathdee SA, Huettner S, et al. Factors associated with HIV seroprevalence among participants enrolling at a needle exchange program. *J Drug Issues*. 2002;32:1125–1138.
35. Darke S. Self-report among injection drug users: a review. *Drug Alcohol Depend*. 1998;51:253–263.
36. Latkin CA, Vlahov D, Anthony JC. Socially desirable responding and self-reported HIV infection risk behaviors among intravenous drug users. *Addiction*. 1993;88:517–526.
37. Menoyo C, Lamikiz E, Zulaika D, et al. The validation of statements by IDUs based on the analysis of blood traces on their used syringes. *AIDS Care*. 1998;10:409–414.
38. Des Jarlais DC, Paone D, Milliken J, et al. Audio-computer interviewing to measure risk behaviour for HIV among injecting drug users: a quasi-randomised trial. *Lancet*. 1999;353:1657–1661.
39. Metzger DS, Koblin B, Turner C, et al. Randomized controlled trial of audio computer-assisted self- interviewing: utility and acceptability in longitudinal studies. *Am J Epidemiol*. 2000;152:99–106.
40. Shrestha S, Strathdee SA, Brahmbhatt H, et al. Short tandem repeat methodology for genotypic identification of single-person versus multi-person use of syringes. *AIDS*. 2000;14:1507–1513.
41. Shrestha S, Smith MW, Beaty TH, et al. Theory and methodology for utilizing genes as biomarkers to determine potential biological mixtures. *Ann Epidemiol*. 2005;15:29–38.
42. Litt M, Luty JA. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardia muscle actin gene. *Am J Hum Genet*. 1989;44:397–401.
43. Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain-reaction. *Am J Hum Genet*. 1989; 44:388–396.
44. Tautz D. Notes on the definition and nomenclature of tandemly repetitive DNA sequences. *EXS*. 1993;67:21–28.
45. Ziegle JS, Su Y, Corcoran KP, et al. Application of automated DNA sizing technology for genotyping microsatellite loci. *Genomics*. 1992;14:1026–1031.
46. Fregeau CJ, Fourney RM. DNA typing with fluorescently tagged short tandem repeats—a sensitive and accurate approach to human identification. *Biotechniques*. 1993;15:100–119.
47. Kimpton CP, Gill P, Walton A, et al. Automated DNA profiling employing multiplex amplification of short tandem repeat loci. *PCR Methods Appl*. 1993;3:13–22.
48. Schlotterer C, Tautz D. Slippage synthesis of simple sequence DNA. *Nucleic Acids Res*. 1992;20:211–215.
49. Hauge XY, Litt M. A study of the origin of 'shadow bands' seen when typing dinucleotide repeat polymorphisms by the PCR. *Hum Mol Genet*. 1993;2:411–415.




Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

50. Weber JL, Wong C. Mutation of human short tandem repeats. *Hum Mol Genet*. 1993;2:1123–1128.
51. Brinkmann B, Klintschar M, Neuhuber F, et al. Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. *Am J Hum Genet*. 1998;62:1408–1415.
52. Vlahov D, Anthony JC, Munoz A, et al. The ALIVE study: a longitudinal study of HIV-1 infection in intravenous drug users: description of methods. *J Drug Issues*. 1991;21:759–776.
53. Nelson K, Galai N, Safaeian M, et al. Temporal trends in the incidence of human immunodeficiency virus infection and risk behavior among injection drug users in Baltimore, Maryland, 1988–1998. *Am J Epidemiol*. 2002;156:641–653.
54. Strathdee SA, Galai N, Safaeian M, et al. Sex differences in risk factors for HIV seroconversion among injection drug users. *Arch Intern Med*. 2001; 161:1281–1288.
55. Latkin CA, Hua W, Davey MA. Exploring the role of needle selling in a drug-using community in Baltimore, Maryland. *J Acquir Immune Defic Syndr*. 2005;38:57–60.
56. Shrestha S, Strathdee SA, Broman KW, et al. Unknown biological mixtures evaluation using STR analytical quantification. *Electrophoresis*. 2006;27:409–415.
57. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*. 1986;42:121–130.
58. Diggle PJ, Liang K, Zieger S. *Analysis of Longitudinal Data*. New York: Oxford University Press; 1996.
59. Shapshak P. Preliminary laboratory studies of inactivation of HIV-1 in needles and syringes containing infected blood using undiluted household bleach. *J Acquir Immune Defic Syndr*. 1994;7:754–759.
60. Gleghorn AA, Doherty MC, Vlahov D, et al. Inadequate bleach contact times during syringe cleaning among injection drug users. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1994;7:767–772.
61. McCoy CB, Rivers JE, McCoy HV, et al. Compliance to bleach disinfection protocols among injecting drug users in Miami. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1994;7:773–776.
62. Bruneau J, Lamothe F, Franco E, et al. High rates of HIV infection among injection drug users participating in needle exchange programs in Montreal: results of a cohort study. *Am J Epidemiol*. 1997;146: 994–1002.
63. Schechter MT, Strathdee SA, Cornelisse PGA, et al. Do needle exchange programmes increase the spread of HIV among injection drug users? An investigation of the Vancouver outbreak. *AIDS*. 1999;13: F45–F51.
64. Remis RS, Bruneau J, Hankins C. Enough sterile syringes to prevent HIV transmission among injection drug users in Montreal. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;18(Suppl):S57–S59.
65. Strathdee SA, Patrick DM, Currie SL, et al. Needle exchange is not enough: lessons from the Vancouver injecting drug use study. *AIDS*. 1997; 11:F59–F65.
66. Peak A, Rana S, Maharjan SH, et al. Declining risk for HIV among injecting drug users in Kathmandu, Nepal—the impact of a harm reduction program. *AIDS*. 1995;9:1067–1070.
67. Kaplan EH, Heimer R. A circulation theory of needle exchange. *AIDS*. 1994;8:567–574.
68. Panda S, Kumar MS, Lokabiraman S, et al. Risk factors for HIV infection in injection drug users and evidence for onward transmission of HIV to their sexual partners in Chennai, India. *J Acquir Immune Defic Syndr*. 2005;39:9–15.
69. Huo D, Bailey SL, Hershow RC, et al. Drug use and HIV risk practices of secondary and primary needle exchange users. *AIDS Educ Prev*. 2005;17: 170–184.
70. Valente TW, Foreman RK, Junge B, et al. Satellite exchange in the Baltimore Needle Exchange Program. *Public Health*. 1999;113:90–96.
71. Koester S, Boothe RE, Shang Y. The prevalence of additional injection-related HIV risk behaviors among injection drug users. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1996;12:202–207.
72. Vlahov D, Khabbaz RF, Cohn S, et al. Incidence and risk factors for human T-lymphotropic virus type II seroconversion among injecting drug users in Baltimore, Maryland, U.S.A. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1995;9:89–96.
73. Denis B, Dedobbeleer M, Collet T, et al. High prevalence of hepatitis C virus infection in Belgian intravenous drug users and potential role of the "cotton-filter" in transmission: the GEMT study. *Acta Gastroenterol Belg*. 2000;63:147–153.
74. Doherty MC, Garfein RS, Vlahov D, et al. Discarded needles do not increase soon after the opening of a needle exchange program. *Am J Epidemiol*. 1997;145:730–737.



**Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.**