

Recovery of Infectious Hepatitis C Virus From Injection Paraphernalia: Implications for Prevention Programs Serving People Who Inject Drugs

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Background. Controlling hepatitis C virus (HCV) transmission among people who inject drugs (PWID) has focused on preventing sharing syringes and drug preparation paraphernalia, but it is unclear whether HCV incidence linked to sharing paraphernalia reflects contamination of the paraphernalia or syringe-mediated contamination when drugs are shared.

Methods. In experiments designed to replicate real-world injection practices when drugs are shared, the residual contents of HCV-contaminated syringes with detachable or fixed needles were passed through the “cookers” and filters used by PWID in preparing drugs for injection and then introduced into a second syringe. All items were tested for the presence of infectious HCV using a chimeric HCV with a luciferase gene.

Results. Hepatitis C virus could not be recovered from cookers regardless of input syringe type or cooker design. Recovery was higher when comparing detachable needles to fixed needles for residue in input syringes (73.8% vs 0%), filters (15.4% vs 1.4%), and receptive syringes (93.8% vs 45.7%).

Conclusions. Our results, consistent with the hypothesis that sharing paraphernalia does not directly result in HCV transmission but is a surrogate for transmissions resulting from sharing drugs, have important implications for HCV prevention efforts and programs that provide education and safe injection supplies for PWID populations.

Keywords. harm reduction; hepatitis C virus; injection drug use; drug paraphernalia; syringes.

Hepatitis C virus (HCV) is among the most common viral infections in the world and is especially common among people who inject drugs (PWID). Prevalence in some populations of PWID is near universal and rarely less than 30% [1, 2]. As with human immunodeficiency virus (HIV), HCV is transmitted within populations of PWID primarily through unsafe injection practices, but unlike HIV, incidence of HCV is often not reduced by increasing access to sterile syringes [3, 4]. This has led researchers and public health officials to explore whether other elements involved in the preparation and injection of illicit drugs play a role in HCV transmission [5, 6]. This hypothesis has been tested, exploring transmission roles for the “cookers” used to dissolve drug for injection, the filters (also referred to as “cottons”) used to filter dissolved drugs, and the water used to prepare drugs or rinse syringes. At least 4 studies have found

epidemiological evidence that although HCV incidence was not associated with sharing syringes, it was associated with sharing other materials used to prepare drugs and apportion them among 2 or more individuals. In the first study, from Seattle, sharing cottons or filters as a single risk factor was significantly associated with HCV incidence with an adjusted risk ratio of 5.9 (95% confidence interval, 1.1–31.7) [7]. In the second study, from Chicago, both sharing cookers and sharing filters were associated with HCV incidence, and in multivariate analysis sharing cookers was associated with incidence with an adjusted hazard ratio of 3.5 (95% confidence interval, 1.3–9.9) [8]. In the third study, from Wales, sharing any injection equipment was associated with incidence with an adjusted risk ratio of 12.7 (95% confidence interval, 1.62–99.6), whereas sharing syringes only was not significantly associated [9]. In the fourth study, from Montreal, receipt and reuse of filters was marginally associated with HCV incidence with an adjusted hazard ratio of 2.15 (95% confidence interval, 0.99–4.67) [10]. Even in some studies in which HCV incidence was associated with sharing syringes, collective use of other materials remained a significant contributor to incidence [11–13]. These findings dovetail with previous studies of risk behaviors for HIV [14, 15], and they have led syringe access and other harm reduction programs that serve PWID to include the provision of clean cookers and filters and

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sterile water among their prevention supplies. However, to date, there have been no epidemiologic reports linking such provision with lowered HCV incidence [16].

There is an alternative hypothesis to explain the association of sharing of cookers and filters with HCV incidence. Observations by ethnographers studying drug injection behaviors have found that sharing of cookers and filters occurs when 2 or more people share the same package(s) of drugs [17–19]. Studies of PWID that have focused on this issue confirm that drug sharing is common among PWID [20–24]. These findings suggest it may not be HCV in shared cookers and filters that leads to transmission, but instead that this kind of sharing is a surrogate for situations in which HCV-discordant injectors share drugs. In this scenario, injectors collectively prepare drugs. If a contaminated syringe was used to add water, dissolve, and apportion the drug, then some of the contents of the contaminated syringe would pass through the cooker and filter and into syringe of the uninfected person. In such a case, the cookers and filters may not even harbor infectious virus, and the distribution of clean ones and warnings about sharing them may have little or no impact on HCV incidence.

We set out to test these 2 competing but not mutually exclusive possibilities. We have developed a microculture assay for HCV propagation in tissue culture that allows us to replicate real-world injection practices in the laboratory and measure the amount and duration of infectivity of HCV in the small volumes found in contaminated syringes [25]. Basing our laboratory procedure on field observations, we prepared syringes to contain the residual amounts of HCV-infected liquid left in syringes after a completed injection and used these contaminated syringes to simulate the preparation of the next injection. In this process, the syringe introduced water to dissolve the drug in a cooker, and the drug solution is drawn up through filters and apportioned to 2 syringes. The contents of the syringes, the cookers, and filters were then entered into the microculture system. This report describes the recovery of infectious HCV from a set of experiments, revealing the ability of each of the items involved in drug preparation and injection to harbor and potentially transmit infectious HCV.

METHODS

Virus and Cells

The experiments described in this report rely on an in vitro microculture system that uses as input virus a chimeric genotype 2a full-length J6/JFH virus that has been modified by the insertion of a *Gaussia princeps* luciferase gene inserted between the p7 and NS2 genes [26–28]. This chimeric Jc1/GLuc2A virus replicates to high copy number in the human hepatoma Huh-7.5 cell line [29]. Previous analysis of sensitivity of the microassay demonstrated that the production of luciferase was strictly linear over a more than 5 log range and directly proportional to the production of infectious virions from <10 to approximately 10^6 tissue culture infectious doses per milliliter [TCID₅₀/mL] [25, 30].

Experimental Procedures

Preparation of Hepatitis C Virus-Contaminated Syringes

We sought to replicate, as closely as possible, the injection scenarios that occur when individuals prepare drugs for injection starting with shared packages (“bags” or “balloons”) of solid drugs. The risk for HCV transmission occurs when the syringe used for adding water to dissolve the drug or apportion the drug once dissolved has been previously used by an individual with an active HCV infection. The practice that produces syringes with the greatest likelihood of HCV contamination is when injectors “boot” [31]. In this common practice, after the drug is injected and needle is still in the vein, the person draws back on the plunger to introduce a little blood into the syringe and reinjects. Injectors describe “booting” as an attempt to get the last remaining drug from the syringe into the vein. When the plunger is depressed after booting, the residual contents of syringe consist almost entirely of blood that is contaminated with HCV if the person doing this is infected. This represents a worst-case scenario, upper bound for HCV contamination within used syringes in that no attempt has been made to disinfect the syringe before it is reused. We replicated this process by loading “input” syringes with the chimeric HCV at a concentration equal to 10 000 TCID₅₀ (Figure 1A). Previous analysis suggests that this concentration is equivalent to 10^6 copies of HCV viral ribonucleic acid [32], which is considered a high viral load in chronically infected patients.

Passage of the Contents of Contaminated Syringes Through Drug Preparation and Injection Paraphernalia

Once contaminated syringes were prepared, we performed experiments to test whether HCV could be recovered from (1) a contaminated input syringe after it had been used as a measuring device to add water for dissolving drugs, (2) from drug preparation paraphernalia (cooker and filter), and (3) the “receptive” syringe that would subsequently inject the dissolved drug. The procedure is depicted in Figure 1B. In brief, water was introduced into the barrel of a contaminated input syringe and expelled into a cooker, and the water was drawn up into a receptive syringe through a cotton filter. The input syringe, cooker, and filter were rinsed with tissue culture medium and introduced into the microculture assay. The water drawn into the second syringe was combined with an equal volume of double-strength medium and introduced into the microculture assay.

This experimental protocol was performed on 10 syringes per experiment, and each experiment was repeated at least 3 times. In each experiment, 2 types of syringes were compared: (1) 1-cc insulin syringes with fixed 27-gauge, half-inch long needles and (2) 1-cc tuberculin syringes with detachable 27-gauge, half-inch long needles. Experiments also compared 2 different types of cookers: both resemble the screw tops of soda bottles, but one is ridged and the other smooth, which is the type generally distributed by harm reduction programs.

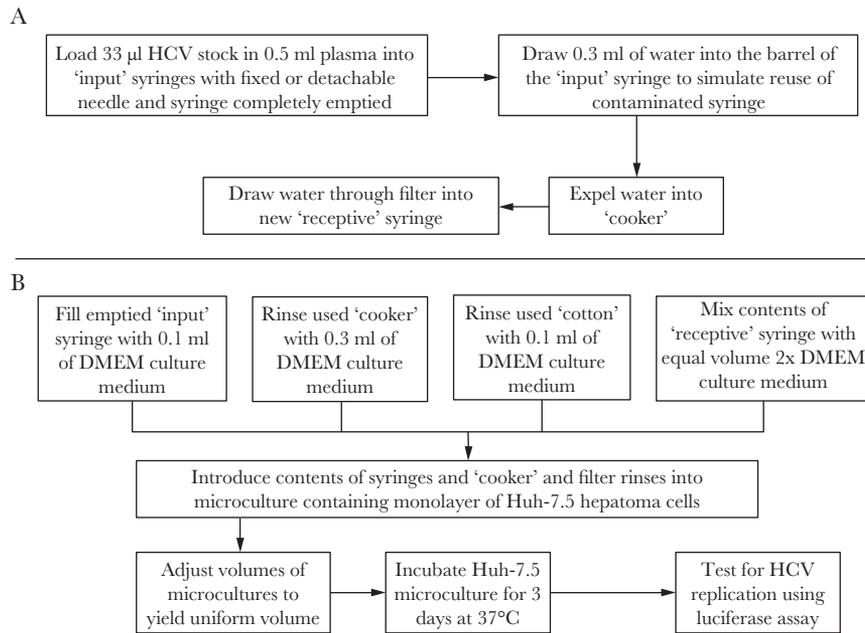


Figure 1. Flow diagram of experiments to determine the recovery of infectious hepatitis C virus (HCV) from drug preparation and injection paraphernalia. (A) The process of producing the syringes and paraphernalia tested in this study is depicted, starting with syringes contaminated with HCV replicating the situation when a syringe previously used by an individual actively infected with HCV is then used to add water to dissolve drugs for injection. The water from the “input” syringes is then passed through a “cooker” and filter and into a “receptive” syringe. (B) The process for testing for potentially infectious HCV in each of the drug preparation and injection items is depicted. The volume of liquid tested is made uniform for all 4 items, and HCV replication after 3 days in microculture is measured according to the procedure described in the Methods and in Paintsil et al [30]. Abbreviation: DMEM, Dulbecco’s modified Eagle’s medium.

A second set of experiments was conducted based on ethnographers’ observations that filters are occasionally saved, pooled, and “beaten” to extract whatever drug residue remains, and the recovered drug is then injected [20, 33, 34]. Such an injection contains material from multiple filters, increasing the likelihood that HCV harbored in filters could yield infectious HCV. We tested 2 scenarios of filter pooling. In the first, 10 filters prepared on the same day as in Figure 1 were combined, beaten, and extracted material was introduced into the microculture assay. However, a more realistic scenario is that pooled filters are stored for periods of time before their contents are extracted. In this experiment, 10 filters stored for up to 10 days were combined, beaten and the extracted material was introduced into the microculture assay.

Analysis of Microculture Assay Results

For the experiments conducted as part of this report, data are presented both as the percentage of syringes, cookers, and filters producing luciferase at levels consistent with viral replication and the mean relative luciferase (relative light units [RLU]) for the positive samples only. Confidence intervals around means and proportions are reported using standard statistics, except for the calculation of confidence intervals when the proportion of tests yielded zero positive results, which was derived using the method of Louis [35]. Pairwise comparisons of recovery frequencies used standard χ^2 with the Fisher exact probability testing.

RESULTS

We used 2 types of syringes—with fixed or detachable needles—because these differ in the amount of fluid retained when the plunger is fully depressed [36–38]. The detachable needle-syringe combination not only retains more fluid but also harbors infectious HCV for much longer periods [25]. We also tested 2 types of metal cookers and single or pooled filters. We have divided the presentation of our results into 3 sections: experiment no. 1 using single filters, experiment no. 2 in which filters were pooled, and a section that combines compatible elements of the 2 experiments.

Experiment No. 1: Hepatitis C Virus Recovery Using Single Filters

The protocol was run 4 times with 10 replicates per run with the ridged cooker and 3 times with 10 replicates per run with the smooth cooker. Thus, a total of 70 sets of injection equipment were tested for the recovery of infectious HCV for each type of syringe in this part of the experiment. The results, depicted in Figure 2, revealed that HCV was recovered more often when using the detachable needle/syringe combinations. When we tested for infectious HCV remaining inside the input syringes that were used to deliver water into the cooker, we failed to recover HCV from the syringes with fixed needles (0 of 70), but we did from 61.4% (43 of 70) of the syringes with detachable needles. This finding is consistent with past studies [25, 39], and the difference was statistically significant ($P \approx 1.57 \times 10^{-17}$ using the

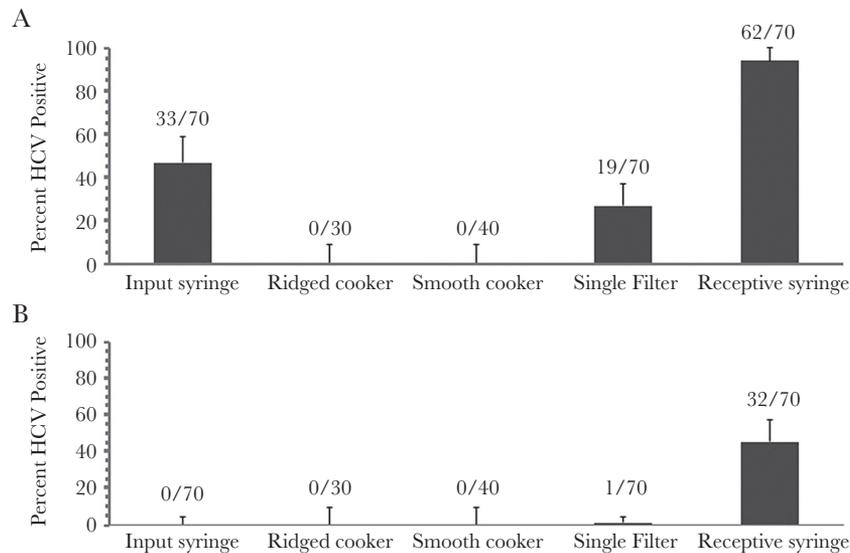


Figure 2. Recovery of infectious hepatitis C virus (HCV) from drug preparation and injection paraphernalia. Syringes were loaded with HCV in plasma and the plunger fully depressed. Water was then drawn into the barrel to simulate the use of a contaminated syringe when preparing drugs for a subsequent injection. (A) Recovery from input syringes, “cookers”, filters, and receptive syringes when the input syringes had detachable needles. (B) Recovery from input syringes, cookers, filters, and receptive syringes when the input syringes had fixed needles.

Fisher exact test). However, virus was successfully passed from both types of syringes to new syringes but at a lower frequency for syringes with fixed needles. Hepatitis C virus was recovered from 94.3% (66 of 70) of syringes when using syringes with detachable needles and 45.7% (32 of 70) of syringes when using syringes with syringes with detachable needles. This difference was statistically significant ($P \approx 1.65 \times 10^{-10}$ using the Fisher exact test).

Recovery of HCV from the drug preparation paraphernalia (cookers and filter) was lower than recovery from syringes. Hepatitis C virus was recovered from 19 of 70 filters (27.1%) when the input syringe adding the water had a detachable needle but only 1 of 70 (1.4%) when the input syringe had a fixed needle. This difference was statistically significant ($P = 4.5 \times 10^{-5}$ using the Fisher exact test). No infectious HCV was recovered from cookers regardless of the type of syringe introducing the contaminated water or the design of the cooker.

In addition to measuring the proportion of items yielding infectious HCV, we quantified the titer of the virus recovered, in terms of relative luciferase transduction activity and expressed at RLU, for those cases in which the RLU exceeded the threshold considered sufficient to indicate active HCV replication (approximately 1000 RLU, based on twice the average negative baseline). In the case of both input syringes and receptive syringes, the average titer was higher when the input into the experiments came from syringes with detachable needles than from syringes with fixed needles.

Experiment No. 2: Hepatitis C Virus Recovery Using Pooled Filters

As noted in the introduction, people may, on occasions when drugs or funds to buy drugs are not available, pool filters and try

to extract drugs from them. We replicated this process by passing water from HCV contaminated input syringes through filters, pooling them, and collecting and testing the material extracted from the pool for the presence of infectious HCV. We prepared pools of 1, 3, 6, and 10 filters using syringes with detachable needles as the input syringes. In addition, because drug injectors often collect and store filters for future extraction, we conducted a time course in which the potentially contaminated filters were extracted immediately (day 0) or stored for 1, 3, or 7 days at room temperature before extraction. Each condition in the time course was tested on 20 replicates on 3 separate occasions.

Filter pooling data are presented in Table 1. On day 0, only a small percentage (1 of 60, 1.7%) of single filters yielded HCV when the input came from syringes with fixed needles, consistent with the data from Experiment no. 1. Increasing the number of filters in the pool increased the proportion, but, even with a pool of 10 filters, only 3 of 60 (5%) of the pools yielded HCV that replicated in culture. We were unable to recover HCV from any of the filter pools once the filters were stored.

In contrast, we were able to recover HCV from a proportion of the input syringes, beginning with 88.3% (53 of 60) without storage, but within 1 week of storage none of the input syringes yielded replicating HCV. In contrast, all receptive syringes, which contained the contaminated material that had passed through the filters, yielded replicating HCV at all times of storage over the week-long time course.

Combined Results of Experiment No. 1 and No. 2

We combined comparable conditions in the 2 sets of experiments, and the results are summarized in Table 2. This allowed

Table 1. Experiment No. 2: Recovery of HCV from Pooled Filters^a

Days of Storage	Item	Filter Pool Size	Number (%) Positive	Average RLU	Standard Deviation
0	Input syringe	–	53 (88.3%)	6758	5175
	Receptive syringe	–	60 (100%)	27 480	10 290
	Filters	1	2 (3.3%)	5200	2674
		3	2 (3.3%)	2589	1576
		6	3 (5.0%)	2038	1064
1	Input syringe	–	29 (48.3%)	3236	2336
	Receptive syringe	–	60 (100%)	14,892	4276
	Filters	1–10	0		
3	Input syringe	–	9 (15%)	1187	202
	Receptive syringe	–	60 (100%)	2893	1135
	Filters	1–10	0		
7	Input syringe	–	0	2249	408
	Receptive syringe	–	60 (100%)		
	Filters	1–10	0		

Abbreviations: HCV, hepatitis C virus; RLU, relative luciferase activity.

^aVirus proliferation from the contents recovered from input syringes with detachable needles, receptive syringes, and filters was measured by determining the RLU from the luciferase gene inserted into the chimeric HCV. For each condition listed below, 60 replications were run, and the average RLU was calculated only for those cases in which the RLU exceeded the threshold indicative of active HCV replication.

us to increase to 130 the total number of input syringes with detachable needles, cookers to which the contents of detachable needles were added, single filters through which the material passed, and receptive syringes. We found that 96 of 130 (73.8%) input syringes, 20 of 130 filters (15.4%), and 122 (93.8%) of the receptive syringes yielded infectious HCV, whereas none of the cookers yielded infectious HCV.

DISCUSSION

Assignment of causality requires validating a set of assumptions; such a set of 9 criteria, set forth by Hill [40], includes biological gradient, plausibility, and experiment. Although the prior set of epidemiological studies [7–10] found strong correlations between the sharing of drug preparation paraphernalia and HCV incidence and established temporality and consistency, those studies could not differentiate between the 2 alternative explanations as to how sharing of paraphernalia other than needles or syringes produced HCV transmission. We have now provided biological evidence that the more compelling explanation

for the association is that sharing of objects associated with the preparation but not the actual injection of drugs is a surrogate for shared injections in which the virus is introduced from a contaminated syringe. We have produced evidence that we can “follow the blood”, and the HCV from a contaminated input syringe ends up in a second receptive syringe, leaving less virus behind in the input syringe and little or no virus in drug preparation paraphernalia.

The conclusion is reinforced if we focus our attention only on the data from syringes with fixed needles, syringes routinely used for the injection of insulin. This style of syringe is most commonly used by PWID in the United States and Canada and is the type overwhelmingly available in pharmacies and supplied by syringe exchange programs. The experimental procedures we describe in this report are equivalent to rinsing the input syringe with water, and, as past work has shown, a single rinse of this type of syringe can greatly reduce the HCV recovery [41]. So it is not surprising that our recovery of HCV from input syringes with fixed needles was negligible. Furthermore,

Table 2. Quantification of HCV Recovery in Experiment No. 1 and No. 2 From Drug Injection and Preparation Paraphernalia^a

Input Syringe	Paraphernalia Item	Positive Tests (%)	Average RLU	Standard Deviation
Syringe with detachable needle	Input syringe	96 (73.8%)	6727	5045
Syringe with detachable needle	Filter	20 (15.4%)	6494	2781
Syringe with detachable needle	Receptive syringe	122 (93.8%)	25 257	11 732
Syringe with attached needle	Input syringe	0	–	–
Syringe with attached needle	Filter	1 (1.4%)	1580	–
Syringe with attached needle	Receptive syringe	32 (45.7%)	4986	3850

Abbreviations: HCV, hepatitis C virus; RLU, relative luciferase activity.

^aThe level of viral proliferation from HCV recovered from items used to prepare and inject drugs was measured by determining the RLU from the luciferase gene inserted into the chimeric HCV used in these experiments and calculated only for those cases in which the RLU exceeded the threshold indicative of active HCV replication. For conditions with fixed needles, the data came from 130 replications; for conditions with fixed needles, the data came from 70 replications.

work over the past decade by a group of researchers, including us, has highlighted the increased risk of bloodborne virus transmission that results from using syringes with detachable rather than fixed needles [25, 36, 37, 39, 42, 43].

Our findings on the retention of HCV in filters differ somewhat from that produced from similar laboratory simulations by Doerrbecker et al [44]. In their experiments, they passed 800 μL of fluid containing approximately 10^5 TCID₅₀/mL through filters. This is far in excess of the 2 to 100 μL of HCV-contaminated fluid that might remain inside a used syringe that is being reused to prepare drugs for injection [25, 36–38]. Although we recovered an average of $23\,107 \pm 13\,870$ RLU from receptive syringes using inputs from syringes with detachable needles, inputs of 10^5 TCID₅₀/mL would yield $>2 \times 10^6$ RLU in our microculture system [25]. Therefore, the experimental protocol of Doerrbecker et al [44] used HCV inputs that are 2 orders of magnitude higher than would occur under “real-world” conditions in which drugs are prepared, shared, and injected, whereas our experimental procedure more closely replicates those conditions.

Our findings on the stability of virus over time in the experiments pooling filters found rapid attenuation of infectivity in the input syringes and filters. This is consistent with our previous findings when titers of HCV are initially low and with findings on the duration of infectivity noted by Doerrbecker et al [25, 45]. A study by Ciesek et al [32], similar to that of Doerrbecker et al [45], used an amount of HCV that was 10-fold higher than we did, so the results are not directly comparable.

As noted by Glass et al [46], once causality in the public health realm is firmly established, appropriate interventions can follow. In this case, it should lead us to reconsider policies, widely adapted by syringe exchange and other harm reduction programs, to provide clean cookers and filters along with sterile syringes when attempting to reduce the transmission of HCV. At a minimum, our findings should compel programs that serve PWID to focus more on the process of drug preparation and injection and less on the preparation paraphernalia. Going further, programs may want to reconsider expanding scarce resources to provide supplies that will do little or nothing to prevent HCV transmission. Given the usual situation of limited financial resources facing syringe exchange and related harm reduction programs, spending money on objects that can have little impact on disease transmission should come to be viewed as profligate. Money spent on cookers and filters would be better spent on giving away more syringes. Because HCV and HIV transmission are more likely if the syringe has a detachable rather than a fixed needle, efforts should focus on providing more syringes with fixed needles. An alternative for people who need syringes with detachable needles is to develop and market reduced dead space syringes [39, 43].

One additional way to improve bloodborne virus prevention efforts is providing guidance and materials to reduce the

chances of using contaminated syringes to prepare or apportion drug. One such piece of drug preparation material could be syringes without accompanying needles that could be used to introduce water into cookers or apportion dissolved drugs. Lacking a needle, such a syringe is unlikely to become contaminated with HCV unless the water source itself was contaminated. Provision of sterile water supplies and training to minimize the commingling of water sources used to prepare drugs and rinse used syringes will do more than the provision of cookers and filters to prevent HCV transmission.

There are 3 significant limitations to our work. First, we are using an in vitro system that is strongly parallel but not identical to the real-world situation, most notably in that it uses a chimeric virus derived from a genotype 2a virus that may not reflect survival and infectivity characteristics of the viruses passed among PWID or when patient-derived viruses are tested in culture. Second, the predominant genotypes among PWID worldwide are genotypes 1 and 3, so it would be useful to replicate our findings with viruses of these genotypes should they become available. If we were to validate our findings using genotype 1 and 3 viruses, it would strengthen the argument about refocusing prevention messaging and the provision of drug preparation and injection supplies more on needles and syringes and less on cookers and filters. Finally, our experiments are replications, reduced realities of real-world situations that are contingent on a host of interacting drug, set, and setting variables [47–50]. Although we have tried to select and replicate a worst-case scenario, we cannot describe the full range of HCV transmission risk that injectors experience.

CONCLUSIONS

Our studies reinforce the need for expanded education efforts and further environmental interventions, such as upscaling distribution of syringes with fixed needles or with reduced dead space to decrease the likelihood for HCV transmission among PWID. These syringes retain less fluid than syringes with detachable needles, and hence less HCV should the person using the syringe be actively infected, and, as previously shown, HCV infectivity persists for a shorter time [25]. Furthermore, as the current study demonstrates, there is less likelihood that shared drug preparation paraphernalia will harbor infectious virus. Given all these benefits, we would advise syringe access and harm reduction education programs to emphasize the distribution of insulin-type syringes with fixed needles and de-emphasize and not expend limited program resources on the distribution of cookers and filters.

Notes

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for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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