

## Hepatitis C Transmission in Injection Drug Users: Could Swabs Be the Main Culprit?

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**Prevention programs for intravenous drug users have proven their efficacy in decreasing human immunodeficiency virus transmission but have limited effect on hepatitis C virus (HCV) contamination. A study was conducted to document the presence of HCV genome (HCV RNA) in 620 items of used injecting paraphernalia collected from representative sites. Using sensitive molecular techniques, HCV RNA was not detected on used filters or water vials and was seldom detected on cups (9%). However, HCV RNA was frequently found on syringe pools (38%) and on swabs (82%) at high titers. Our investigation, which was conducted in real injecting settings, highlights swabs as a potential source for HCV transmission.**

Among the 170 million persons infected with the hepatitis C virus (HCV) worldwide, most were contaminated through unsafe injections. This route of infection also explains the high prevalence of HCV infection in persons who inject drugs (PWID). Strikingly, the prevalence of HIV infections in PWID never reaches values as high as those observed for HCV, and HCV infections occur earlier during the addiction period [1]. Many elements favor higher transmissibility of HCV than of human immunodeficiency virus (HIV). First, the median replication level in untreated patients fluctuates around 6 log<sub>10</sub> IU/mL, with most chronic carriers being unaware of their infective status and consequently not following any safe behaviors. Second, virus survival in the environment may be

more prolonged than initially thought. Indeed, 2 recent studies using different approaches have convincingly demonstrated that HCV is relatively resistant in the environment, providing some clues to explain its high transmissibility [2, 3].

Recent works have underlined the good efficacy of prevention programs in reducing blood-borne virus transmission in PWID. Many programs included provide not only sterile needles and syringes but also other materials that are shared such as cookers, filters, water, and swabs [4]. Yet all reports led to the same conclusion. Although very efficient in reducing HIV spread, these programs had disappointing results in terms of HCV transmission [5, 6]. High HCV loads coupled with relative HCV resistance could explain the potential role of injecting paraphernalia, other than syringes and needles, in the spread of HCV in PWID [7]. In light of these data and evidence that injecting practices may not be as standardized and “safe” as expected, we conducted a large study to document the presence of HCV RNA in used injecting equipment from several representative locations in Paris and its surroundings.

### Methods

Used injecting equipment was collected in collaboration with several community associations from Aulnay-sous-Bois (FIRST) and Paris (GAIA, STEP, and SAFE). These associations are in charge of harm-reduction programs that distribute single-use injecting paraphernalia (needles, syringes, filters, cups, sterile water, alcohol swabs, and cotton pads), either from a specific location or through vending machines and individualized counseling.

Two campaigns were organized to collect used materials. The first campaign (study 1) targeted only storefront offices of associations involved in needle-exchange programs, with the primary purpose of collecting equipment from HCV-infected drug users, that is, users who knew their HCV-positive status. The second campaign (study 2) did not include any specific criterion regarding HCV infection, was performed among local community associations, and targeted also automated vending and collecting machines. Drug users were made aware of the study and asked to return their used materials as soon as possible. Items were collected from automated vending machines on a daily basis. Equipment was stored at 4°C and then frozen at −20°C until processed.

All material was sorted according to type (syringes, cups, filters, swabs, water vials) and pooled into groups of 10 items. Syringes were thoroughly rinsed with 1 mL of sterile water per pool, that is, 1 mL of water was used to rinse 10 syringes from the same pool. Each pool of cups was also rinsed thoroughly

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with 1 mL of sterile water. To eliminate any floating residue, cup rinsing liquid was then spun for 10 minutes at 7000 rpm and further filtered through a 0.45- $\mu$ m filter (Minisart; Sartorius Stedim Biotech). One milliliter of water from each used water vial was pooled together. To completely soak all collected used swabs, up to 10 mL of water was used for each pool of 10 swabs. Soaked swabs were spun for 10 minutes at 3500 rpm, and the supernatant, which consisted of  $\sim$ 1 mL of liquid, was collected. Each pool of 10 filters was treated according to the process used for swabs and soaked in 2 mL of water before centrifugation and collection of supernatants.

HCV RNA detection was performed in the laboratory (Virology Laboratory, Pitié-Salpêtrière Hospital) using an Abbott HCV RealTime assay (Abbott Molecular Diagnostics), according to procedures specified by the manufacturer. Briefly, 1 mL of rinsing water obtained from each pool was separately treated. Nucleic acids were purified on an M2000sp (Abbott) instrument with detection by real-time polymerase chain reaction (PCR) on the M2000rt system. The limit of quantification, as defined by the manufacturer, is 12 IU/mL for an input volume of 0.5 mL. Importantly, an internal control was added to each specimen to validate the entire process from nucleic acid collection to PCR amplification. Lack of internal control amplification leads to a noninterpretable result that is considered invalid and not taken into account in the final prevalence report. Before all samples were tested, 1 specimen of each material was artificially contaminated with a known amount of HCV RNA to validate the process.

### Results

The first study was designed to assess feasibility, and 160 used pieces of equipment were collected from suspected HCV-infected PWID. Fifty swabs or cotton pads, 20 cups, 20 vials, 10 filters, and 60 syringes were pooled by type and analyzed for HCV RNA contamination. For almost half (43.8%) of all pooled materials, HCV RNA could be detected. The highest prevalence of HCV RNA was detected in alcohol swabs and cotton pads (80%) (Figure 1).

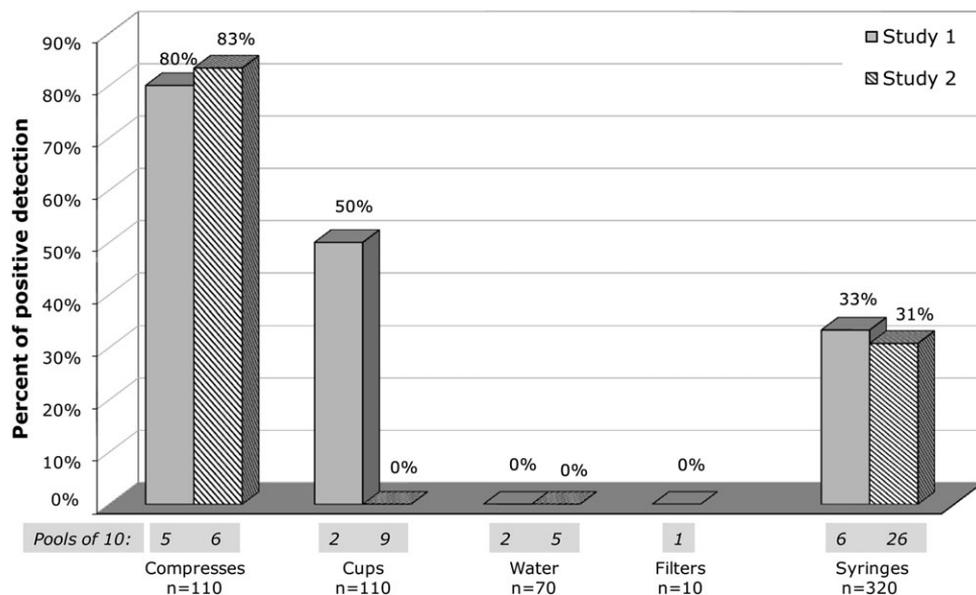
The second study was designed to validate our findings and observe the situation in a real-life setting without selecting for presumably HCV-positive individuals. Material collection sites were chosen to target consumption of different drugs and to target users at different social levels. Used items were pooled together according to their type and their collection site. In this second study, 60 swabs, 90 cups, 50 vials of water, and 260 syringes were collected. HCV RNA was detected in 28% of pooled materials; 83% of the pools obtained from swabs were HCV RNA positive. As observed elsewhere, viral loads were highest ( $>3 \log_{10}$  IU/mL) within swab pools (Table 1). Although HCV RNA was commonly found in syringes, viral loads were usually lower, with values ranging from 12 to 890 IU/mL. It is noteworthy that all syringe pools ( $n = 5$ ) collected at a specific

site (Maison de la Radio, Paris) were positive for HCV RNA, whereas only 3 of 21 (14%) from other locations were contaminated. HCV RNA was never detected on the few filter pools tested or on collected water vials. Overall, 62 pools of equipment were analyzed and only 1 HCV RNA test was not validated, owing to inhibition of internal control amplification by interfering substances.

### Discussion

This is the largest reported study to look at the presence of HCV RNA on used materials collected at multiple drug consumption sites. Although detection of HCV RNA does not necessarily mean infectious particles, the relatively high viral loads (slightly above  $3 \log_{10}$  IU/mL in some pools) observed on some items are striking. Indeed, recent work by Ciesek et al demonstrated the absence of correlation between viral infectivity and RNA copy numbers, particularly after a few days of storage at room temperature [3]. However, detection of high viral concentration in a pool indicates that at least 1 piece of equipment was contaminated and could have been a potential source of transmission if shared shortly after usage. The most striking finding was the high rate of HCV detection in pools of swabs (82%), particularly when compared with the rather low rate of contaminated syringes (32%). For both materials, residual blood was often macroscopically visible, and it was expected that many syringes would test HCV RNA positive. Recently, it has been shown that HCV could survive and be potentially infectious in syringes stored for up to 63 days under different conditions; the main culprit for HCV transmission was clearly designated [2]. However, not only were swabs more often found to contain HCV RNA but levels of contamination on swabs were also in most cases 10 times higher (median, 412 IU/mL; range, 12–4932) than those on syringes (median, 12 IU/mL; range, 12–890). One explanation for this discrepancy could be that residual amount of blood on some swabs was greater than that in syringe bodies. Thus the consequence is also a higher chance for PWID to be contaminated through sharing of a tainted swab rather than a tainted syringe. Another possible explanation, which may lead to a bias in our study, is the ritual habit of rinsing a syringe before exchange or reuse [8]. Our results are in agreement with those of Crofts et al who also describe the presence of HCV RNA in  $>50\%$  of both syringes ( $n = 20$ ; 70%) and swabs ( $n = 9$ ; 67%) [9]. However, our approach is slightly different, because we performed HCV RNA detection in pools of 10 items, using a very robust and sensitive quantification assay for all HCV genotypes [10, 11].

It is noteworthy that blood was macroscopically visible on both alcohol swabs and cotton pads, even though swabs should be used before injection and pads after. Injections are often performed by a third party, and it is conceivable that a swab is used to stop bleeding before being used by a second person [8, 12, 13]. These observations highlight an inappropriate use of



**Figure 1.** Percentage of hepatitis C virus RNA detection on collected used injecting equipment. Each piece of equipment was tested in a pool of 10 for presence of HCV RNA, with use of real-time polymerase chain reaction analysis. Numbers of tested pools and items are indicated on the x-axis.

these items. Thus, transmission is possible if a pad tainted with up to 5 log<sub>10</sub> IU/mL of HCV, after taking into account the dilution factor resulting from pooling, is shared [8]. The chaotic and rushed atmosphere of the injection setting, where swab sharing and mixing could take place, is also an important factor that should be considered [14].

The relative heterogeneous distribution of HCV-tainted materials according to the site of collection is interesting and validates our effort to include drug users who take different drugs and come from different social substrata. In a recent study, Nefau and colleagues, using highly sensitive methods, were able to characterize the drugs found in residual contents of used syringes [15, 16]. In one location where all syringes were HCV RNA positive, the main drug found in syringes was cocaine. Considering this information, it is tempting to conclude that HCV prevalence in cocaine users may be higher than in other populations, at least in areas of Paris that were explored in this study. Interestingly, this population seems more refractory to education programs and is possibly less aware of at-risk behaviors.

This study has at least 2 limitations. The first results from the use of HCV RNA detection instead of a true infectiousness measurement. However, true infection methods that rely on cell culture are too complex and not sensitive enough to be directly applied to such material. Second, we chose to constitute pools of material in order to test a large number of them. It is conceivable that the true prevalence of contaminated material was overestimated if a single piece of equipment, among the pool of 10, contained high concentrations of viruses. Yet the relative high viral load observed, particularly for swab pools, indicates that almost 5 log<sub>10</sub> IU are present on a single swab, which is certainly sufficient to transmit an infectious dose of virus. Absence of virus detection in water vials could be linked to a dilution effect, because many vials were almost full at the time of collection. Thus, firm conclusions cannot be drawn from our study regarding filters (only a few were tested) and water, and the potential risk of contamination through sharing of these materials should not be obscured. Other studies should be conducted to confirm our findings and address other shared paraphernalia more specifically.

**Table 1. Details of Hepatitis C Virus (HCV) RNA Detection and Quantification, According to Equipment Collected**

Material	HCV RNA study 1, % (no.)		HCV RNA study 2, % (no.)	
	Detected	Quantified <sup>a</sup>	Detected	Quantified <sup>a</sup>
Cotton swabs	80 (4/5)	50 (2/4) [3969 IU/mL]	83 (5/6)	80 (4/5) [901 IU/mL]
Cups	50 (1/2)	(0/1)	0 (0/9)	...
Water vials	0 (0/2)	...	0 (0/5)	...
Filters	0 (0/1)	...	...	...
Syringes	33 (2/6)	100 (2/2) [ 221 IU/mL]	31% (8/26)	25 (2/8) [ 919 IU/mL]

Abbreviation: NA, not applicable.

<sup>a</sup> Quantified among samples that were HCV RNA positive. Mean levels are shown in brackets.

In summary, our investigation, which was performed in real injecting settings, highlights 2 main sources for HCV contamination, namely, syringes and swabs; other collected items were less frequently contaminated. These findings should help raise awareness and target prevention programs to at-risk behaviors and specific populations. To improve their efficacy, programs should include stronger messages to prevent sharing not only syringes but also swabs. Action or communication to clearly specify that alcohol swabs are for use only before injection and cotton pads are for use only after injection would also be necessary. Other strategies to explore could include distribution of swabs impregnated with disinfectants active against microorganisms. By decreasing HCV infectivity, transmission may be then reduced even if the same swab is used before and after injection or shared between users.

## Notes

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## References

1. Jauffret-Roustide M, Emmanuelli J, Quaglia M, et al. Impact of a harm-reduction policy on HIV and hepatitis C virus transmission among drug users: recent French data—the ANRS-Coquelicot Study. *Subst Use Misuse* **2006**; 41:1603–21.
2. Paintsil E, He H, Peters C, Lindenbach BD, Heimer R. Survival of hepatitis C virus in syringes: implication for transmission among injection drug users. *J Infect Dis* **2010**; 202:984–90.
3. Ciesek S, Friesland M, Steinmann J, et al. How stable is the hepatitis C virus (HCV)? Environmental stability of HCV and its susceptibility to chemical biocides. *J Infect Dis* **2010**; 201:1859–66.
4. Gillies M, Palmateer N, Hutchinson S, Ahmed S, Taylor A, Goldberg D. The provision of non-needle/syringe drug injecting paraphernalia in the primary prevention of HCV among IDU: a systematic review. *BMC Public Health* **2010**; 10:721.
5. Mehta SH, Astemborski J, Kirk GD, et al. Changes in blood-borne infection risk among injection drug users. *J Infect Dis* **2011**; 203: 587–94.
6. Palmateer N, Kimber J, Hickman M, Hutchinson S, Rhodes T, Goldberg D. Evidence for the effectiveness of sterile injecting equipment provision in preventing hepatitis C and human immunodeficiency virus transmission among injecting drug users: a review of reviews. *Addiction* **2010**; 105:844–59.
7. Mathei C, Shkedy Z, Denis B, et al. Evidence for a substantial role of sharing of injecting paraphernalia other than syringes/needles to the spread of hepatitis C among injecting drug users. *J Viral Hepat* **2006**; 13:560–70.
8. Treloar C, Laybutt B, Jauncey M, et al. Broadening discussions of “safe” in hepatitis C prevention: a close-up of swabbing in an analysis of video recordings of injecting practice. *Int J Drug Policy* **2008**; 19:59–65.
9. Crofts N, Caruana S, Bowden S, Kerger M. Minimising harm from hepatitis C virus needs better strategies. *BMJ* **2000**; 321:899.
10. Bortoletto G, Campagnolo D, Mirandola S, et al. Comparable performance of TMA and real-time PCR in detecting minimal residual hepatitis C viraemia at the end of antiviral therapy. *J Clin Virol* **2011**; 50:217–220.
11. Pyne MT, Konnick EQ, Phansalkar A, Hillyard DR. Evaluation of the Abbott investigational use only RealTime hepatitis C virus (HCV) assay and comparison to the Roche TaqMan HCV analyte-specific reagent assay. *J Clin Microbiol* **2009**; 47:2872–8.
12. O’Connell JM, Kerr T, Li K, et al. Requiring help injecting independently predicts incident of HIV infection among injection drug users. *J Acquir Immune Defic Syndr* **2005**; 40:83–8.
13. Bryant J, Treloar C. Initiators: an examination of young injecting drug users who initiate others to injecting. *AIDS Behav* **2008**; 12: 885–90.
14. Hagan H, Pouget ER, Des Jarlais DC. A systematic review and meta-analysis of interventions to prevent hepatitis C virus infection in people who inject drugs. *J Infect Dis* **2011**; 204:74–83.
15. Karolak S, Nefau T, Bailly E, Solgadi A, Levi Y. Estimation of illicit drugs consumption by wastewater analysis in Paris area (France). *Forensic Sci Int* **2010**; 200:153–60.
16. Nefau T, Karolak S, Levi Y, Duplessy C, Bara JL. Analyse des résidus de drogues dans les seringues usagées. *Le Courrier des Addictions* **2011**; 13:10–2.