

Criteria Grid
Best Practices and Interventions for the Prevention and Awareness of Hepatitis C

Best Practice/Intervention:	Binka M. et al. (2015) Disinfection of syringes contaminated with hepatitis C virus by rinsing with household products. Open Forum Infect Dis, 23;2(1): ofv017.			
Date of Review:	February 8 th , 2016			
Reviewer(s):	Christine Hu			
Part A				
Category:	Basic Science <input type="checkbox"/> Clinical Science <input checked="" type="checkbox"/> Public Health/Epidemiology <input type="checkbox"/> Social Science <input type="checkbox"/> Programmatic Review <input type="checkbox"/>			
Best Practice/Intervention:	Focus: Hepatitis C <input checked="" type="checkbox"/> Hepatitis C/HIV <input type="checkbox"/> Other: _____ Level: Group <input checked="" type="checkbox"/> Individual <input type="checkbox"/> Other: _____ Target Population: <u>people who inject drugs (PWID) disinfect used syringes with household products</u> Setting: Health care setting/Clinic <input checked="" type="checkbox"/> Home <input type="checkbox"/> Other: _____ Country of Origin: <u>USA</u> Language: English <input checked="" type="checkbox"/> French <input type="checkbox"/> Other: _____			
Part B				
	YES	NO	N/A	COMMENTS
<i>Is the best practice/intervention a meta-analysis or primary research? Please go to Comments section.</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Primary research: to determine the effectiveness of household products in disinfecting HCV contaminated syringes
<i>The best practice/intervention shows evidence of "scale up" ability</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
<i>The best practice/intervention shows evidence of transferability</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	The study may be transferable to individuals disinfecting syringes using household products.
<i>The best practice/intervention shows evidence of adaptation</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	

<i>Do the methodology/results described allow the reviewer(s) to assess the generalizability of the results?</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	The study used a genotype 2a laboratory clone of HCV which prevents the generalizability of the results of all population of HCV infected patients.
	YES	NO	N/A	COMMENTS
<i>Are the best practices/methodology/results described applicable in developed countries?</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
<i>Are the best practices/methodology/results described applicable in developing countries?</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	The study is applicable to all other countries given that they have easy access to the household products included in the research.
<i>The best practice/intervention has utilized a program evaluation process</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
<i>Consultation and feedback with community has taken place</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
<i>The best practice/intervention is sensitive to gender issues</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
<i>The best practice/intervention is sensitive to multicultural and marginalized populations</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Study is mainly intended for people who inject drugs.
<i>The best practice/intervention is easily accessed/available electronically</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Full article can be accessed at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4438897/
<i>Is there evidence of a cost effective analysis? If so, what does the evidence say? Please go to Comments section</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
<i>How is the best practice/intervention funded? Please go to Comments section</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	This study was funded by the National Institutes of Health/National Institute on Drug abuse.
<i>Is the best practice/intervention dependent on external funds?</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
<i>Other relevant criteria:</i> _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	- The study found that bleach was the most effective product at eliminating residual HCV infectivity from tuberculin and insulin syringes

				<ul style="list-style-type: none">- The study also showed that 70% isopropanol, Dawn Ultra kitchen sink detergent and 3% hydrogen peroxide were effective at eliminating residual HCV infectivity in low void volume insulin syringes after 1 rinse <p>Limitation</p> <ul style="list-style-type: none">- Researchers were unable to test most of the household products at undiluted concentrations- HCV-spiked plasma were used instead of HCV-spiked blood, which could affect the results
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Disinfection of Syringes Contaminated With Hepatitis C Virus by Rinsing With Household Products

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Background. Hepatitis C virus (HCV) transmission among people who inject drugs (PWID) is associated with the sharing of injection paraphernalia. People who inject drugs often “disinfect” used syringes with household products when new syringes are unavailable. We assessed the effectiveness of these products in disinfecting HCV-contaminated syringes.

Methods. A genotype-2a reporter virus assay was used to assess HCV infectivity in syringes postrinsing. Hepatitis C virus-contaminated 1 mL insulin syringes with fixed needles and 1 mL tuberculin syringes with detachable needles were rinsed with water, Clorox bleach, hydrogen peroxide, ethanol, isopropanol, Lysol, or Dawn Ultra at different concentrations. Syringes were either immediately tested for viable virus or stored at 4°C, 22°C, and 37°C for up to 21 days before viral infectivity was determined.

Results. Most products tested reduced HCV infectivity to undetectable levels in insulin syringes. Bleach eliminated HCV infectivity in both syringes. Other disinfectants produced virus recovery ranging from high (5% ethanol, 77% ± 12% HCV-positive syringes) to low (1:800 Dawn Ultra, 7% ± 7% positive syringes) in tuberculin syringes.

Conclusions. Household disinfectants tested were more effective in fixed-needle syringes (low residual volume) than in syringes with detachable needles (high residual volume). Bleach was the most effective disinfectant after 1 rinse, whereas other diluted household products required multiple rinses to eliminate HCV. Rinsing with water, 5% ethanol (as in beer), and 20% ethanol (as in fortified wine) was ineffective and should be avoided. Our data suggest that rinsing of syringes with household disinfectants may be an effective tool in preventing HCV transmission in PWID when done properly.

Keywords. bleach; HCV transmission; hepatitis C virus; people who inject drugs; syringe disinfection.

Approximately 150 million people are chronically infected with hepatitis C virus (HCV) around the world [1]. Chronic infection with HCV can result in health complications such as chronic liver disease, cirrhosis of the liver, and hepatocellular carcinoma [1, 2]. Hepatitis

C virus is transmitted by exposure to infected blood during blood transfusions and the reuse of equipment for intravenous injections [1–3]. Sexual and perinatal transmissions of HCV are not efficient [2, 3]. Widespread screening of blood supplies for HCV, human immunodeficiency virus (HIV), and other pathogens has reduced HCV transmission by blood transfusion in developed countries [2]. People who inject drugs (PWID) now constitute one of the largest groups of people infected with HCV worldwide [2, 4, 5]. With a global population of 11 to 21 million, HCV prevalence rates among PWID are largely higher than 40% in many areas, compared with average HIV prevalence rates of below 20% within the same population [2, 5].

High rates of HCV transmission in PWID persist due to unsafe injection practices within this group [2, 6]. Hepatitis C virus transmission in PWID has been linked

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to the sharing of syringes, needles, and other injection paraphernalia; practices that continue despite extensive harm reduction efforts [2, 6, 7]. Hepatitis C virus incidence rates are highest in younger PWID, aged 15 to 24 years old, who are more likely to share drug injection paraphernalia within their social networks [2, 8]. Furthermore, recent studies have shown that active attempts at HCV serosorting among PWID is compromised by inaccurate knowledge of their injecting partners' HCV infection status [6, 9, 10].

Human immunodeficiency virus and HCV can survive on surfaces for more than 4 weeks at room temperature [11–14]. Human immunodeficiency virus is stable in syringes for up to 42 days, whereas HCV is stable for at least 1 day in insulin syringes with fixed needles and up to 63 days in tuberculin syringes with detachable needles at room temperature [15, 16]. Harm reduction programs have encouraged the use of bleach, rubbing alcohol, and dishwashing detergent, to rinse used injecting equipment when new supplies are unavailable to PWID [17–19]. People who inject drugs also clean their syringes with various readily available beverages, including cola, wine, beer, and vodka in an attempt to prevent disease transmission [20]. Studies have shown that many of these household products, with the exception of bleach, are ineffective at disinfecting HIV-contaminated syringes [20, 21]. These products also have varying levels of success at inactivating HCV dried on surfaces or kept in suspension [12–14, 22]. However, information regarding the efficacy of household products in disinfecting HCV-contaminated syringes is very limited.

In this report, we close this knowledge gap by determining the ability of different household products to disinfect HCV-contaminated insulin and tuberculin syringes by using our previously established microculture assay [13, 15].

MATERIALS AND METHODS

Virus and Cells

The Jc1/GLuc2A reporter virus, a derivative of the chimeric genotype 2a full length J6/JFH virus with a *Gaussia princeps* luciferase gene inserted between the p7 and NS2 genes, was used in this study [23–25]. The protocol for virus preparation has been reported previously [13, 15].

The human hepatoma Huh-7.5 cell line, which is highly permissive for HCV infection [26], was cultured as adherent monolayers in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Omega Scientific, Tarzana, CA) and 1 mM nonessential amino acids (Invitrogen) with 5% CO₂ at 37°C [13, 15].

For experiments involving toxic concentrations of disinfectants, Huh-7.5 cells were also cultured in the presence of a 1:1 v/v mixture of MicroSpin S-400 HR sephacryl column eluates (GE Healthcare, Freiburg, Germany) and 2× DMEM (EMD

Millipore, Darmstadt, Germany) supplemented with 20% heat-inactivated fetal calf serum (Omega Scientific) and 2 mM nonessential amino acids (Invitrogen Life Technologies).

Syringes

People who inject drugs use different types of syringes for injecting drugs including low void volume syringes that retain ~2 μL and high void volume syringes that retain 10-fold or more residual liquid after use [27, 28]. We used 2 types of syringes for our experiments: 1 mL U-100 insulin syringes with 27-gauge 0.5-inch attached needles (Terumo Medical, Somerset, NJ), representing the low void volume syringes, and 1 mL tuberculin syringes with 27-gauge 0.5-inch detachable needles (Terumo Medical), representing syringes with high void volumes.

Household Products

People who inject drugs rinse their used syringes with household products or alcoholic beverages in an attempt at disinfection [20]. We tested the ability of these liquids to disinfect low and high void volume syringes contaminated with HCV and compared virus recovery to that obtained from syringes rinsed with distilled water or left unrinsed.

The following household products were tested: ethanol (AmericanBio, Natick, MA), isopropanol (AmericanBio), Clorox Concentrated Regular Bleach (8.25% sodium hypochlorite; The Clorox Company, Oakland, CA), Lysol Multi-Surface Pourable Cleaner (Pacific Fresh Scent; Reckitt Benckiser, Parsippany, NJ), Dawn Ultra (Original Scent; The Procter and Gamble Company, Cincinnati, OH), and Hydrogen Peroxide (3% H₂O₂; McKesson, Richmond, VA).

The following alcoholic beverages were tested: Corona Extra Beer (4.6% alcohol; Crown Imports, Chicago, IL), Harvey's Bristol Cream Sherry (17.5% alcohol; Harvey's Import Company, Deerfield, IL), and Dubra Vodka (40% alcohol; Dubra Distillers Product Company, Clifton, NJ).

Cytotoxicity of Different Household Products on Huh-7.5 Cells

To control for the effect of these liquids on our microculture system, we first determined their cytotoxic effects to the Huh-7.5 cells. In brief, Huh-7.5 cells were seeded at 1.5×10^4 cells per well in 96-well plates in 100 μL of cell culture media. The next day, 1 mL insulin and 1 mL tuberculin syringes were rinsed once with 500 μL of different concentrations of the various products. The syringes were then flushed with 100 μL of cell culture media, which was used to replace an equal volume of cell culture media that was aspirated from the cell culture wells. The cells were incubated overnight at 37°C, and cell growth was determined with the alamarBlue assay (Invitrogen Life Technologies) as directed by the manufacturer. First, the flushed medium was aspirated from the wells and replaced with 50 μL of fresh culture medium. The 5 μL alamarBlue reagent was then added to the cells, and the plates were wrapped in aluminum foil and incubated at 37°C for 4 h. Cell growth was

determined as a function of relative fluorescence measured at 530 nm excitation and 590 nm emission (Synergy HT Plate Reader; BioTek, Winooski, VT). Five syringes were tested per condition and the experiment was repeated 3 times.

For household products that proved toxic to the Huh-7.5 cells at the concentrations tested, the 100 μ L flushed syringe contents were filtered through sephacryl S-400 HR columns to trap smaller inhibitory molecules (GE Healthcare) according to the manufacturer's instructions. Eluates of 100 μ L were then mixed with 100 μ L of 2 \times DMEM (EMD Millipore) prior to addition to the Huh 7.5 cells. Cell growth was again determined with the alamarBlue assay described above. Five syringes were tested per condition and the experiment was repeated 3 times.

Viability of Human Immunodeficiency Virus in Syringes After Rinsing With Different Household Products

We slightly modified the protocol used in our previous studies [13, 15] to test for residual HCV infectivity after rinsing syringes with various household products. In brief, 1 mL insulin and 1 mL tuberculin syringes were loaded with HCV-spiked plasma and rinsed once or multiple times with 500 μ L of the liquids at different concentrations. The syringes were then stored at 4°C, 22°C, or 37°C for up to 21 days, after which they were flushed with 100 μ L of cell culture media and introduced into cell culture on Huh-7.5 cells seeded the previous day in 96-well plates at 1.5×10^4 cells per well. The cells were then incubated with the flushed virus for 5 h at 37°C, after which they were washed once with 100 μ L sterile phosphate-buffered saline ([PBS] Invitrogen Life Technologies, NY), and 100 μ L of fresh cell culture media was added. Cells were incubated for 3 days at 37°C before viral supernatant was harvested and lysed with 20 μ L of lysis buffer (Promega, Madison, WI). Viral infectivity was determined as function of relative luciferase units (RLU) as measured with a luciferase assay kit (Promega) and a luminometer (Synergy HT, BioTek, VT). Ten syringes were tested per condition and the experiment was repeated at least 3 times.

When testing household products that had proven cytotoxic to Huh-7.5 cells, the syringe contents post-rinsing were flushed with 100 μ L of medium, filtered through the sephacryl columns, and mixed with 100 μ L 2 \times DMEM prior to addition to the plated cells. After a 5-hour incubation at 37°C, cells were washed once with 100 μ L sterile PBS, and 100 μ L fresh cell culture media was added. Viral supernatants were harvested after 3 days of incubation at 37°C, and infectivity was measured as described above. Ten syringes were tested per condition and the experiment was repeated 3 times. The results are presented as the percentage of HCV-positive syringes yielding an RLU value above a pre-established cutoff (\pm standard error of the mean) and the mean residual infectivity, measured in RLU, calculated with data only from the syringes yielding RLU values above the cutoff (\pm standard deviation). The cutoff value of 1000 RLU was set at 2 times the background RLU measurements.

RESULTS

Cytotoxic Effects of Household Products on Huh-7.5 Cells

Before assessing the effectiveness of different household products at disinfecting 1 mL insulin and 1 mL tuberculin syringes, we determined their effect on Huh-7.5 cell growth by using alamarBlue assays. For a majority of the products, the syringe contents after rinsing 1 mL insulin syringes were not toxic to the Huh-7.5 cells (Figure 1A). Cell growth was comparable with that of the distilled water control with the exception of undiluted bleach, 3% hydrogen peroxide, and Lysol, which killed the cells, reducing fluorescence to levels comparable to a cell-free control. Bleach dilution of 1:10 reduced cell growth to 43% of the positive control (Figure 1A). Therefore, bleach, 3% hydrogen peroxide, and Lysol were diluted with water before rinsing syringes to restore cell growth to acceptable levels (Figure 1A). Kitchen sink detergent is generally not used undiluted, so we determined that the highest concentration at which it could be used in 1 mL insulin syringes without reducing cell growth was at a 1:300 dilution in distilled water.

Due to the higher residual volume of disinfectant in the 1 mL tuberculin syringes, more household products were toxic to the Huh-7.5 cells in 1 mL tuberculin syringes than in 1 mL insulin syringes (Figure 1A and B). Both 70% ethanol and 70% isopropanol, in addition to undiluted bleach, 3% hydrogen peroxide, and Lysol, were toxic to the Huh-7.5 cells; completely eliminating cell growth (Figure 1B). Their further dilution in water was required to maintain cell growth after rinsing 1 mL tuberculin syringes. With 1 mL tuberculin syringes, Dawn Ultra was diluted further at 1:800 dilution in distilled water to maintain cell growth (Figure 1B).

Most of the disinfectants are meant to be applied at higher concentrations than were ultimately used to disinfect the 1 mL tuberculin syringes. To get closer to the standard concentrations used for disinfection, we increased the concentrations and added a filtration step. Under these conditions, we reduced the cytotoxic effects of 70% ethanol, 70% isopropanol, and 1:10 bleach to restore cell growth to levels comparable with the distilled water control (Figure 1C). However, the cytotoxic effects of undiluted bleach, 3% hydrogen peroxide, and Lysol were not overcome by filtration, and further dilution in water was required before rinsing and filtration to maintain cell growth (Figure 1C). We observed a slight decrease in cell growth upon filtration of syringe contents through the sephacryl columns. This could be the result of incubating the cells with "2 \times DMEM-column eluate" mixtures with suboptimal concentrations somewhere between 1 \times and 2 \times .

Effect of Household Products on Hepatitis C Virus Stability in Contaminated Syringes

We determined the effect of rinsing HCV-contaminated syringes with various household products on HCV recovery from the residual contents of the rinsed syringes. All products tested were effective at eliminating residual infectivity in HCV-contaminated

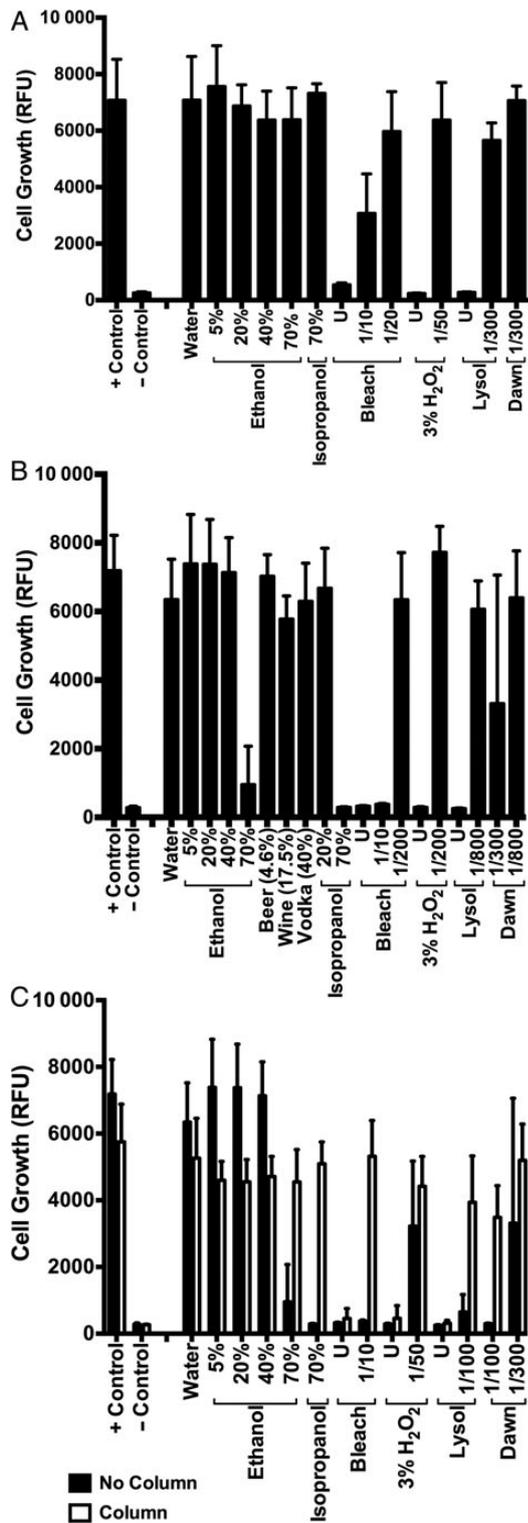


Figure 1. Effect of household products on Huh-7.5 cell growth. Different products—water, ethanol, isopropanol, bleach, hydrogen peroxide (H₂O₂), Lysol, and Dawn Ultra—were tested at varying concentrations for their effect on Huh-7.5 cell growth after rinsing 1 mL (A) insulin and (B) tuberculin syringes. (C) At higher product concentrations, tuberculin syringe contents were filtered through S-400 HR sephacryl columns before contact with the cells. Each data point represents the average relative fluorescence units (RFU) ± standard deviation from 3 experiments. HCV, hepatitis C virus; U, undiluted.

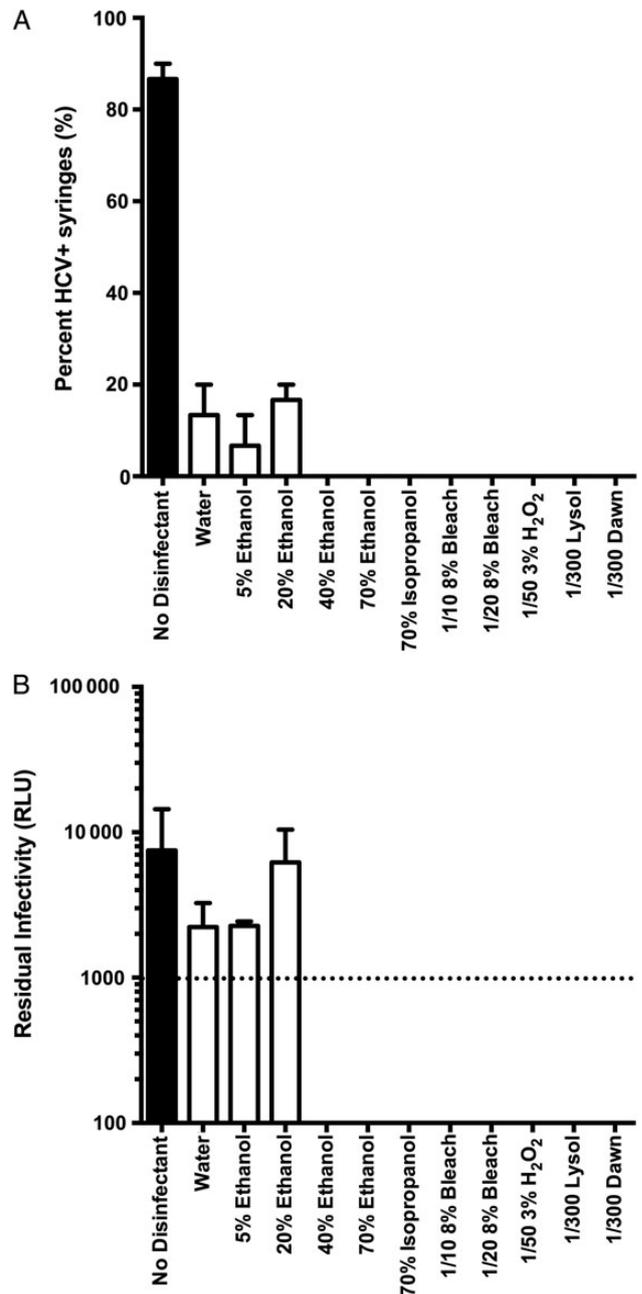


Figure 2. Survival of hepatitis C virus (HCV) in 1 mL insulin syringes after rinsing once with different household products. The 1 mL insulin syringes were loaded with HCV-spiked plasma and rinsed once with the indicated household products. The remaining syringe contents post-rinsing were flushed out with 100 μ L of cell culture media and exposed to the Huh-7.5 cells. (A) Percentage of HCV-positive syringes and (B) residual HCV infectivity after rinsing. Black bars represent the positive control with no rinsing (“No Disinfectant”). Each data point represents the average relative luciferase units (RLU) ± standard deviation or percent positive ± standard error of the mean from 3 experiments.

1 mL insulin syringes with the exception of 5% ethanol (7% ± 7% HCV-positive syringes), 20% ethanol (17% ± 3% positive syringes), and water (13% ± 7% positive syringes) (Figure 2A). Little

difference was apparent in the infectivity of recovered virus, measured in RLU, from HCV-positive syringes after rinsing with these 3 agents (Figure 2B).

Tuberculin syringes, on average, had 7 times greater HCV infectivity levels than the insulin syringes as measured in RLU (compare “No Disinfectant”, Figures 2B and 3B). Residual infectivity after the rinsing of HCV-contaminated 1 mL tuberculin syringes was comparable for water (8224 ± 6720 RLU), 5% ethanol ($10\,010 \pm 7976$ RLU), and 20% ethanol (6142 ± 4081 RLU) (Figure 3B). The 1:800 Dawn Ultra and vodka produced $7 \pm 7\%$ and $7 \pm 3\%$ of HCV-positive syringes, respectively, and residual infectivity of 1292 ± 314 RLU and 1356 ± 375 RLU, respectively (Figure 3A and B). None of the HCV-contaminated 1 mL tuberculin syringes was positive for HCV after rinsing with 1:200 bleach (Figure 3A). Rinsing tuberculin syringes with 5% ethanol was equivalent to rinsing with beer ($10\,010 \pm 7976$ RLU compared with 9748 ± 9135 RLU residual infectivity, respectively), whereas rinsing with 20% ethanol was equivalent to rinsing with fortified wine (6142 ± 4081 RLU compared with 9594 ± 7044 RLU residual infectivity); this process suggests no virucidal activity beyond the concentration of ethanol in these beverages. A wide range of HCV recovery was observed in the rinsed 1 mL tuberculin syringes; ranging from high (water, $93\% \pm 7\%$ positive syringes; 5% ethanol, $77\% \pm 12\%$ positive syringes) to medium (40% ethanol, $27\% \pm 9\%$ positive syringes; 1:200 3% hydrogen peroxide, $23\% \pm 7\%$ positive syringes) and low (1:800 Lysol, $7\% \pm 3\%$ positive syringes; 1:800 Dawn Ultra, $7\% \pm 7\%$ positive syringes) (Figure 3A). Multiple rinses were required at these concentrations to reduce residual HCV infectivity to background levels (Figure 4A and B).

Filtering the flushed contents of 1 mL tuberculin syringes through sephacryl columns resulted in HCV recovery at levels similar to those obtained in 1 mL insulin syringes (data not shown).

In both 1 mL insulin and tuberculin syringes, water, 5% ethanol, and 20% ethanol were consistently shown to be the least effective “disinfectants” (Figures 2A and 3A). Therefore, we assessed HCV stability in 1 mL tuberculin syringes after rinsing with these 3 products and storage at 4°C , 22°C , or 37°C for up to 21 days (Figure 5). In accordance with previously published data [15], HCV stability upon storage postrinsing was temperature-dependent for all 3 products tested (Figure 5A–F). The percentage of HCV-positive syringes varied slightly for the different ethanol concentrations, but the overall pattern of HCV recovery was the same: $4^{\circ}\text{C} > 22^{\circ}\text{C} > 37^{\circ}\text{C}$ (Figure 5A, C, and E). In general, residual infectivity decreased after 7 days of storage with no infectivity detected by 21 days (Figure 5A, C, and E).

DISCUSSION

Bleach has been promoted for decades, as part of harm reduction efforts, as a suitable disinfectant for used syringes and injection

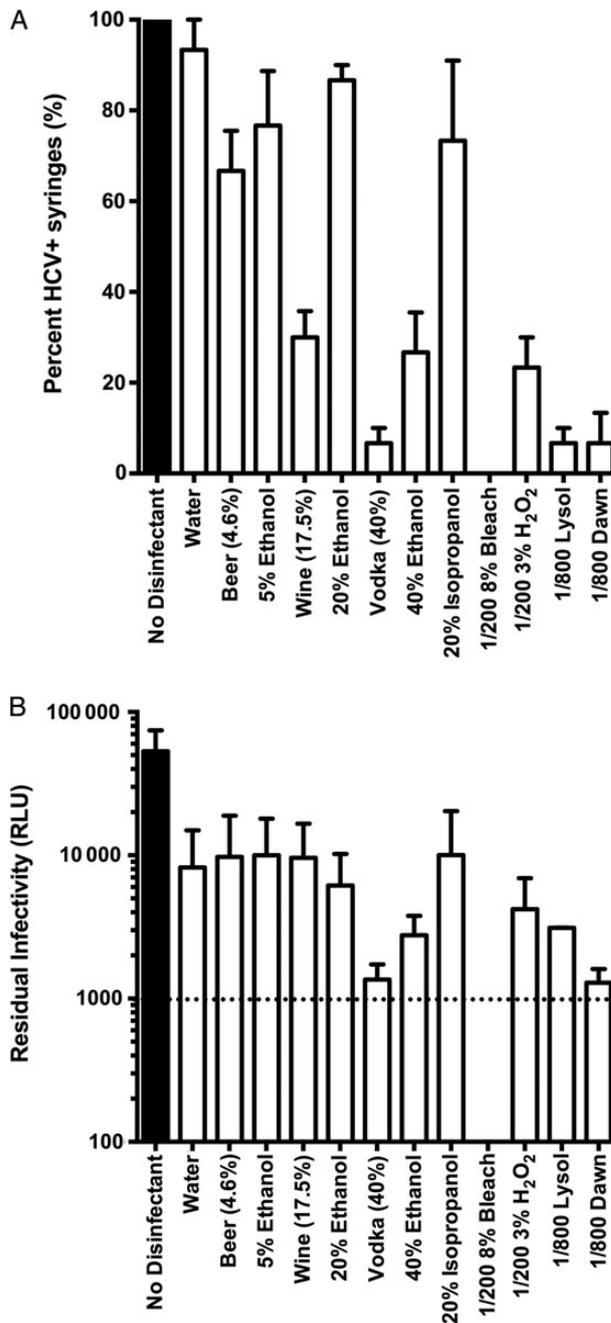


Figure 3. Hepatitis C virus (HCV) stability in 1 mL tuberculin syringes after rinsing once with different household products. The 1 mL tuberculin syringes were loaded with HCV-spiked plasma and rinsed once with the indicated household products. The remaining syringe contents postrinsing were flushed out with 100 μL of cell culture media and exposed to the Huh-7.5 cells. (A) Percentage of HCV-positive syringes and (B) residual HCV infectivity after rinsing. Black bars represent the positive control with no rinsing (“No Disinfectant”). Each data point represents the average relative luciferase units (RLU) \pm standard deviation or percent positive \pm standard error of the mean from 3 experiments.

paraphernalia among PWID [19, 21, 29, 30]. In our study, bleach was the most effective product at eliminating residual HCV infectivity in both tuberculin and insulin syringes. This result is

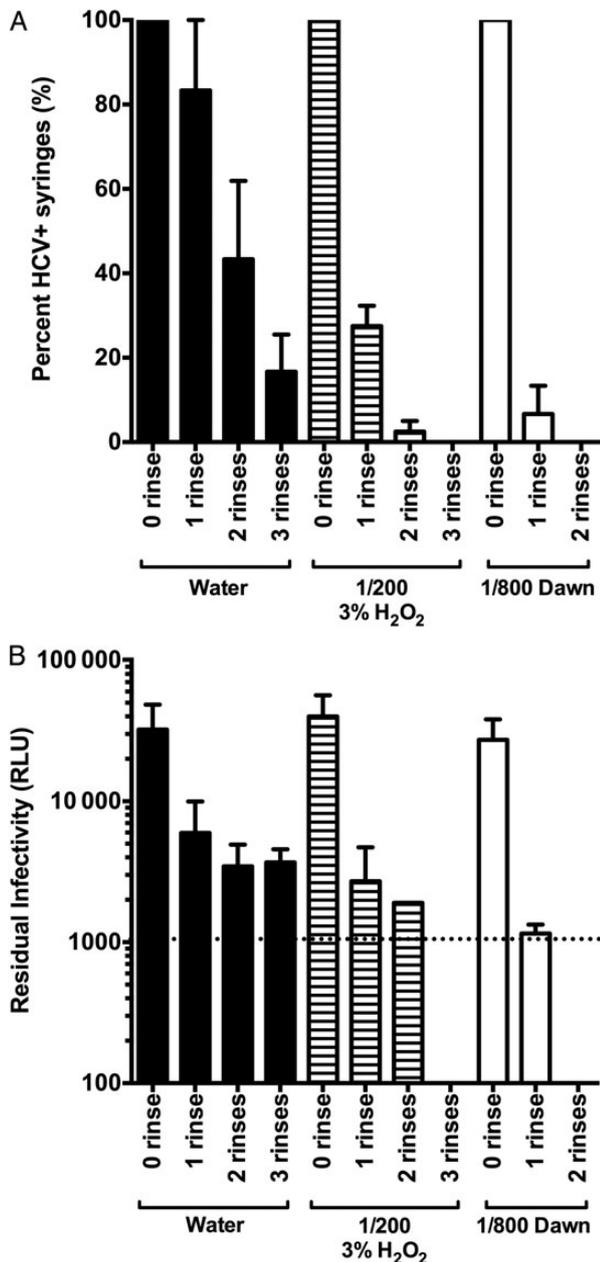


Figure 4. Hepatitis C virus (HCV) survival in 1 mL tuberculin syringes after rinsing multiple times with different household products. The 1 mL tuberculin syringes were loaded with HCV-spiked plasma and rinsed several times with the indicated household products. The remaining syringe contents postrinsing were flushed out with 100 μ L of cell culture media and exposed to the Huh-7.5 cells. (A) Percentage of HCV-positive syringes and (B) residual HCV infectivity after rinsing. Each data point represents the average relative luciferase units (RLU) \pm standard deviation or percent positive \pm standard error of the mean from at least 3 experiments.

consistent with other studies that reported the effectiveness of bleach in eliminating residual HIV infectivity in contaminated syringes [20, 21]. As such, bleach may be the best disinfectant for decontaminating used syringes, to prevent both HCV and HIV transmission, when new syringes are unavailable.

There remains some controversy as to whether there is a correlation between PWID rinsing their syringes with bleach and the reduction of HIV and HCV transmission. Nevertheless, PWID may choose not to rinse their syringes with bleach for a number of reasons, including the fact that multiple rinses with bleach is damaging to the syringes and needles [20, 29, 30]. Approximately 20 rinses with undiluted bleach cause significant damage to both syringes and needles [20]. However, it takes almost 3 times as many rinses with rubbing alcohol, hydrogen peroxide, and diluted kitchen sink detergent to cause damage to syringes and needles [20]. Therefore, PWID may prefer rubbing alcohol, hydrogen peroxide, and diluted kitchen sink detergent to bleach for rinsing of syringes and needles.

When interviewed, a majority of the PWID had rubbing alcohol (70% isopropanol), kitchen sink detergent, and hydrogen peroxide nearby when they last injected [20]. Our study showed that 70% isopropanol, Dawn Ultra kitchen sink detergent (1:300 dilution in water), and 3% hydrogen peroxide (1:50 dilution in water) were, indeed, effective at eliminating residual HCV infectivity in low void volume insulin syringes after 1 rinse. We can infer from the fact that 3% hydrogen peroxide and Lysol were effective with 1 rinse of insulin syringes at suboptimal concentrations that they would be just as effective undiluted.

We were unable to test most of the household products at their undiluted concentrations in the high void volume 1 mL tuberculin syringes due to increased cytotoxicity. A majority of these products were highly diluted to reduce their toxic effects in our assay. Under these conditions, none of the household products, with the exception of 1:200 bleach, was able to eliminate residual HCV infectivity. Multiple rinses with 1:200 3% hydrogen peroxide and 1:800 Dawn Ultra were required to reduce residual HCV infectivity in the tuberculin syringes to undetectable levels. Based on these results, we can surmise that fewer rinses may be required to decontaminate the syringes when rinsed with the higher, undiluted concentrations of hydrogen peroxide, rubbing alcohol, and other disinfectants. However, given the limitations of our assay, we are unable to offer definitive recommendations. Water, 5% ethanol (as in beer), and 20% ethanol (as in fortified wine) were ineffective at inactivating HCV in both low and high void volume syringes and should be avoided. Three rinses with water were not enough to eliminate HCV infectivity in 1 mL tuberculin syringes; this indicates that numerous rinses may be required when beverages, other than those with at least 40% ethanol, are used to disinfect syringes. Taken together, our findings suggest that hydrogen peroxide, rubbing alcohol, Lysol, and kitchen sink detergent may be suitable alternatives to bleach in high and low void volume syringes, if high concentrations are used and if syringes are rinsed several times.

In agreement with previous data [15], HCV stability in tuberculin syringes was temperature-dependent in syringes rinsed with water, 5% ethanol, and 20% ethanol. Hepatitis C virus

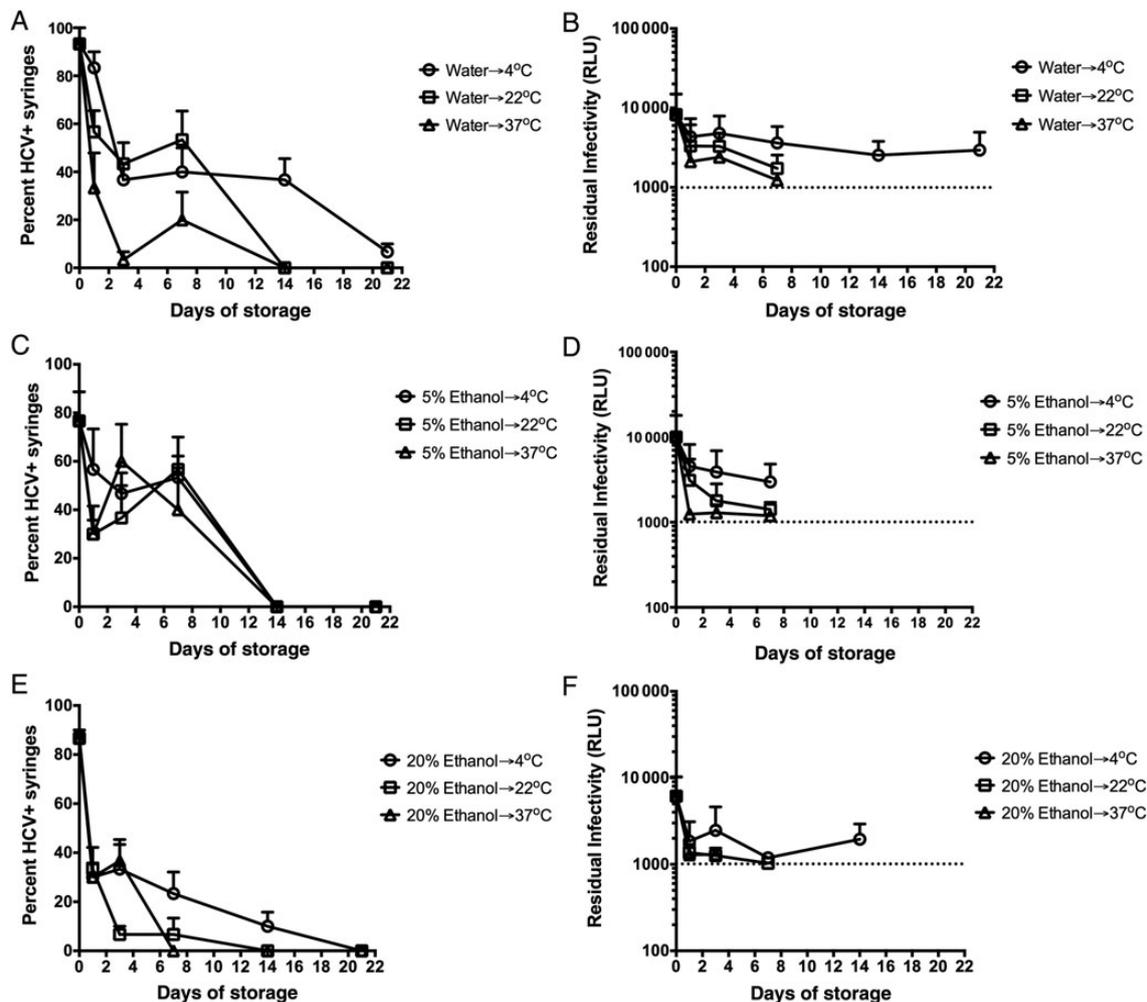


Figure 5. Effect of temperature on hepatitis C virus (HCV) survival in 1 mL tuberculin syringes after rinsing once with water, 5% ethanol, and 20% ethanol. The 1 mL tuberculin syringes were loaded with HCV-spiked plasma, rinsed once with water, 5% ethanol, and 20% ethanol and stored at 4°C, 22°C, or 37°C for up to 3 weeks. Syringe contents were then flushed and exposed to Huh-7.5 cells. Percentage of HCV-positive syringes after rinsing with (A) water, (C) 5% ethanol, and (E) 20% ethanol. Residual HCV infectivity after rinsing with (B) water, (D) 5% ethanol, and (F) 20% ethanol. Each data point represents the average relative luciferase units (RLU) ± standard deviation or percent positive ± standard error of the mean from 3 experiments.

stability increased as temperature decreased (4°C > 22°C > 37°C), and this stability was independent of ethanol concentration. These findings have implications for the places where PWID store their syringes and injection paraphernalia, such that storage in the heat of the summer would be more likely to inactivate the virus than storage in an air-conditioned location. In the same way, storage in the glove compartment of a car in the summer would inactivate the virus more quickly than in winter. We hope to investigate the effect of rinsing syringes with disinfectants after removing them from storage, because some PWID may rinse their stored syringes right before their reuse.

Our study has a number of limitations. First, we used a genotype 2a laboratory clone of HCV, which may not be representative of primary isolates of this and other genotypes, especially the genotype 1b virus that is most common among infected people in

many locations [31]. However, HCV clones of different genotypes have identical responses to temperature changes and, therefore, can be expected to react similarly to other physical stimulus [32]. Second, we used HCV-spiked plasma, as opposed to HCV-spiked blood, which could affect the results because other blood components may alter the interaction of the virus and disinfectant. Finally, we were not able to test many of the disinfectants at their recommended concentrations, which prevents us from definitively establishing their effectiveness against the virus. Despite these limitations, our results allow us to draw inferences about the potential activity of household products against HCV in syringes based on their activity in diluted form. Furthermore, the similarity between our findings and those obtained from comparable work done with HIV, another enveloped virus, further bolsters the validity of our data [20, 21].

CONCLUSIONS

This study sought to establish the virucidal activity of a variety of commercially available household products in HCV-contaminated syringes. To our knowledge, this is the first study to examine HCV stability in low and high void volume syringes after rinsing with common household products. We found bleach to be best at disinfecting HCV-contaminated syringes even when used at concentrations far below 1:10 dilution recommended by the Centers for Disease Control and Prevention for working with blood products [33]. When bleach is unavailable, other products could be used, but only with multiple rinses. Although we do not advocate syringe rinsing as an alternative to using new syringes or needles, rinsing and disinfection may be a better option than simply reusing injecting equipment when new ones are unobtainable. However, it should be noted that syringe disinfection must be done properly for the benefits to be achieved. As the HCV prevalence rate continues to rise within PWID population, further research alongside fieldwork needs to be done to identify more practical solutions for PWID that reduce HCV transmission while being functional under “street conditions.” The alternatives for HCV control among PWID, such as the scaled-up combination of harm reduction and effective treatment for addiction [34] or universal access to directly acting HCV antiviral medications [35], are simply not available to most PWID. In their absence, broad dissemination of information about the utility and proper use of household disinfectants can assist in the control of the HCV epidemic among PWID.

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