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Testing Strategy To Identify Cases of Acute Hepatitis C Virus (HCV) Infection and To Project HCV Incidence Rates[▽]

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Surveillance for hepatitis C virus (HCV) is limited by the challenge of differentiating between acute and chronic infections. In this study, we evaluate a cross-sectional testing strategy that identifies individuals with acute HCV infection and we estimate HCV incidence. Anti-HCV-negative persons from four populations with various risks, i.e., blood donors, Veterans Administration (VA) patients, young injection drug users (IDU), and older IDU, were screened for HCV RNA by minipool or individual sample nucleic acid testing (NAT). The number of detected viremic seronegative infections was combined with the duration of the preseroconversion NAT-positive window period (derived from analysis of frequent serial samples from plasma donors followed from NAT detection to seroconversion) to estimate annual HCV incidence rates. Projected incidence rates were compared to observed incidence rates. Projected HCV incidence rates per 100 person-years were 0.0042 (95% confidence interval [95% CI], 0.0025 to 0.007) for blood donors, 0.86 (95% CI, 0.02 to 0.71) for VA patients, 39.8 (95% CI, 25.9 to 53.7) for young IDU, and 53.7 (95% CI, 23.4 to 108.8) for older IDU. Projected rates were most similar to observed incidence rates for young IDU (33.4; 95% CI, 28.0 to 39.9). This study demonstrates the value of applying a cross-sectional screening strategy to detect acute HCV infections and to estimate HCV incidence.

It is estimated that hepatitis C virus (HCV) infects nearly 4 million persons in the United States (3) and 130 million persons worldwide (19). In the United States, chronic HCV infection is the primary cause of end-stage liver disease resulting in liver transplantation (2). Transfusion-transmitted HCV has been virtually eliminated in the developed world as a result of routine screening of blood products by using progressively more sensitive antibodies and, since 1999, minipool nucleic acid testing (NAT) (25, 47). However, new infections still occur at high rates in other at-risk populations, especially injection drug users (IDU). Worldwide, the prevalence of HCV infection among IDU ranges from 25% to 80% (14, 21, 22, 27, 34, 45), and the incidence among younger IDU ranges from 9% to 38% per year (13, 16, 23, 37, 49).

Monitoring HCV infection has principally been limited to serosurveys detecting HCV-specific antibodies (anti-HCV), using enzyme immunoassays (EIAs) and confirmatory recombinant immunoblot assays (RIBAs). However, these cannot differentiate between acute, recent, chronic, and resolved infections, distinctions which would be very useful for HCV surveillance. Recent infection represents the period from exposure through early seroconversion, with acute infection comprising the viremic preseroconversion phase (the viremic preseroconversion "window period"). Although most acutely infected individuals (60 to 80%) will become chronic carriers of the virus, infection is self-limited in an average of 26% of individuals who spontaneously resolve viremia (2, 36). Very few data are available about the rate of acute HCV infection (anti-HCV-negative/RNA-positive infections) in various populations. Such data could inform surveillance, help to identify and target interventions to high-risk populations, and assist in identifying cases for early treatment (10).

Traditionally, epidemiologic evaluation of incidence has required the establishment and follow-up of large uninfected cohorts. These prospective studies are expensive to conduct; high-risk populations, such as IDU, are challenging to follow, and biased incidence estimates may result. In this study, we evaluate a testing algorithm, based on cross-sectional laboratory analysis, which allows detection of acute HCV infection and estimation of incidence. This strategy makes use of the RNA-only phase of HCV infection, when acutely infected individuals have high levels of HCV plasma viremia (detectable by NAT) prior to antibody seroconversion. A single blood specimen obtained from an individual determined to be antibody negative (via an HCV EIA) but RNA positive (via HCV NAT) represents a case of acute infection. Using this strategy and the duration of the viremic preseroconversion window

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TABLE 1. HCV antibody prevalence by risk population

Population	Specimen collection dates (mo/yr)	Median age (yr)	n	Seroprevalence (% anti-HCV positive)
Blood donors	1/00–12/05		4,620,687	0.1
VA patients	7/98–3/99	58	1,032	18
Young IDU	1/00–9/06	22	1,095	40[a]
Older IDU				
≥10 yr of injecting	1998–2000	45	1,210	92
<10 yr of injecting	1998–2000	35	391	82
Total			1,601	89

[a] Of 1,095 persons tested, 146 were anti-HCV positive by EIA 3 and 289 were anti-HCV positive by EIA 2 (435/1,095 = 40%).

period defined by these assays, we demonstrate that the proportion of acutely infected individuals can be used to estimate HCV incidence in various populations. Secondarily, for a subsample of high-risk IDU with acute HCV infection, we compare the use of pooled or diluted sample NAT screening to individual sample NAT screening for incident case detection.

(Data presented in this paper were previously presented in partial form at the 13th Conference on Retroviruses and Opportunistic Infections, Denver, CO, February 2006 [43].)

MATERIALS AND METHODS

Sample populations. Specimens were obtained from a large population of blood donors and from three high-risk populations, including Veteran Administration (VA) clinic patients and patients from two studies (younger and older) of IDU. Demographic data for each of these populations are outlined in Table 1. All of the participating studies had protocols reviewed and approved from their respective institutions, and participants in each had consented to have blood samples tested for secondary studies. The UCSF Institutional Review Board approved the protocol for this study.

Specimens from blood donors were tested for HCV RNA according to a donor screening protocol using minipools of 16 donations, with reactive pools resolved to individual RNA-positive specimens (detailed below) (47). Blood donations were screened in parallel for HCV antibodies (EIA 3.0, with confirmation by RIBA 3) and RNA. Donations with evidence of acute infection were confirmed by supplemental RNA testing (PCR), and donors were followed up to confirm seroconversion as detailed elsewhere (47). VA specimens were obtained from a cross-sectional HCV seroprevalence study that sampled one of every three patients undergoing routine phlebotomy at an outpatient clinical laboratory (8). Cross-sectional specimens were obtained from an ongoing study of young IDU recruited by street outreach-based methods in San Francisco (22) from January 2000 through September 2006, and if they were anti-HCV negative, the patients were enrolled in longitudinal follow-up (23). All were <30 years, reported IDU in the prior 6 months, and spoke English. The median duration of injecting was 4 years. Specimens from older IDU were obtained using the following two sampling strategies: (i) retrospective examination of stored plasma specimens collected from 1987 to 1998 from inner-city San Francisco Bay Area IDU who had been injecting drugs for <10 years, and (ii) cross-sectional analysis of stored plasmas obtained from a cohort of street-recruited IDU sampled every 6 months, who had been using drugs for >10 years (median duration, 22 years of IDU) (31, 34).

Antibody-negative/RNA-positive testing strategy. The antibody-negative/RNA-positive testing strategy requires (i) a sensitive antibody EIA (HCV 3.0 EIA; Ortho Diagnostic Systems, Raritan, NJ) and (ii) a sensitive, qualitative HCV RNA assay. In the present study, we used the discriminatory HCV transcription-mediated amplification (dHCV TMA) assay component of the Procleix HIV-1/HCV assay, developed by Gen-Probe Inc., San Diego, CA, and marketed by Chiron Blood Screening (now a division of Novartis), Emeryville, CA. The dHCV TMA assay has been approved by the FDA as a stand-alone qualitative HCV RNA assay for detection of acute HCV infection and for confirmation of viremia in seroreactive specimens (HCV Aptima assay; Gen-Probe Inc.). The dHCV TMA assay can detect 12.1 (95% confidence interval [95% CI], 11.1 to 13.2) copies/ml of HCV RNA (50% detection probability) in the recommended 0.5-ml specimen input. Due to this high sensitivity, pooled testing is able to detect HCV RNA in the majority of cases of acute infection, despite the dilution factor inherent in minipool NAT screening (11, 18, 35). Blood donations were tested for anti-HCV and RNA by Blood Systems Laboratories (Tempe, AZ, and Bedford, TX) as part of routine donor screening, as required by the FDA. All other testing was performed at Chiron/Novartis, Emeryville, CA.

The testing algorithm to detect acute HCV infections is shown in Fig. 1. Plasma specimens collected from the populations (except for blood donors) were initially screened for anti-HCV using HCV 3.0 EIA (which is validated for use with both plasma and serum specimens), with confirmation by RIBA 3.0 testing (Chiron Corporation). Seropositive specimens were considered to have come from persons exposed to HCV who had either chronic or resolved HCV infections and were eliminated from further testing (blood donor specimens were tested for anti-HCV and RNA concurrently). Anti-HCV-negative specimens were tested for HCV RNA by dHCV TMA. For all populations, except young IDU, the pooled sample RNA screening strategy was used. First, to increase throughput and reduce costs, EIA-negative specimens were combined into minipools of 16 (blood donors) or 10 to 16 (high-risk populations) specimens, which were tested for HCV RNA. All members comprising an RNA-negative pool were deemed uninfected and required no further testing. RNA-positive pools were resolved by testing the composite specimens undiluted (with the specified 0.5-ml specimen input), using dHCV TMA. For each individual pool member found to be RNA positive, infection status was confirmed by (i) verifying the anti-HCV-negative status by using repeat HCV 3.0 EIA and/or RIBA 3.0 testing and (ii) testing backup aliquots of frozen plasma to confirm RNA positivity and to rule out contamination as a source for RNA reactivity. Confirmed anti-HCV-negative/HCV RNA-positive results were used to estimate HCV incidence. Among young IDU, all HCV RNA testing was conducted using individual specimens (not pooled), with follow-up confirmatory procedures as detailed above for RNA-positive specimens.

Pooled testing compared to individual testing strategy for detection of HCV RNA. We compared the sensitivity of the pooled testing strategy to that of the individual sample testing strategy, using the anti-HCV-negative/RNA-positive specimens from young IDU, including both acute infection samples identified during cross-sectional screening and those from follow-up visits from these acute infection cases that also tested anti-HCV negative/RNA positive. Separately aliquoted specimens were tested undiluted and at 1:10 and 1:100 dilutions (in negative plasma), comparable to the minipool testing strategy, and results were compared to the yields of RNA detection from the individual testing strategy.

Derivation of the preseroconversion window period for use in the acute HCV testing/incidence estimation algorithm. To estimate the duration of the viremic preseroconversion window period, we analyzed data on frequent (one or two donations per week) serial donations from 58 source plasma donors identified as anti-HCV negative/RNA positive while in the process of serially donating plasma units to Bayer Corporation (Raleigh, NC). These donors were enrolled into an FDA-approved clinical trial of the pooled sample NAT screening system, with subsequent follow-up through seroconversion (50). The majority (n = 43) provided serial donations that included at least one RNA-negative and anti-HCV-negative sample, followed by multiple RNA-positive, antibody-negative samples, and finally one or more antibody-positive samples. In a few cases (n = 3), seroconversion was not documented owing to limited follow-up; hence, seroconversion is not bounded in time for these donors. There were also some first-time donors (n = 12), enrolled based on an initial viremic seronegative donation, who lacked prior donations for evaluation of the date of exposure; hence, RNA conversion is also not bounded in time in all cases. Since the plasma donors used in this analysis were not tested daily, the exact date of RNA or antibody positivity cannot be ascertained exactly. Therefore, for this analysis of interval-censored data (33), we assumed that viremia could have occurred on any day between the last RNA-negative donation and the first RNA-positive donation, and seropositivity could have occurred on any day between the last antibody-negative donation and the first antibody-positive donation. The estimated window period represents the mean interval of time from RNA positivity to antibody positivity. This estimate of the duration window period depends slightly on an assumed distribution. While a normal distribution is theoretically not plausible, other plausible distributions all yielded estimates very similar to the normal distribution-based estimate. Thus, the normal distribution-based estimate is presented. A Kaplan-Meier-like plot (adapted for interval-censored data) was generated to graphically present the estimated time from development of viremia detectable by pooled sample NAT to EIA 3 seroconversion (33).

Estimating HCV incidence and comparison with observed incidence. Projected incidence rates were calculated from cross-sectional acute infection data with the following formula: $I = (n_{vsn}/N_{sn}) \times (365/T) \times (100)$, where I is the

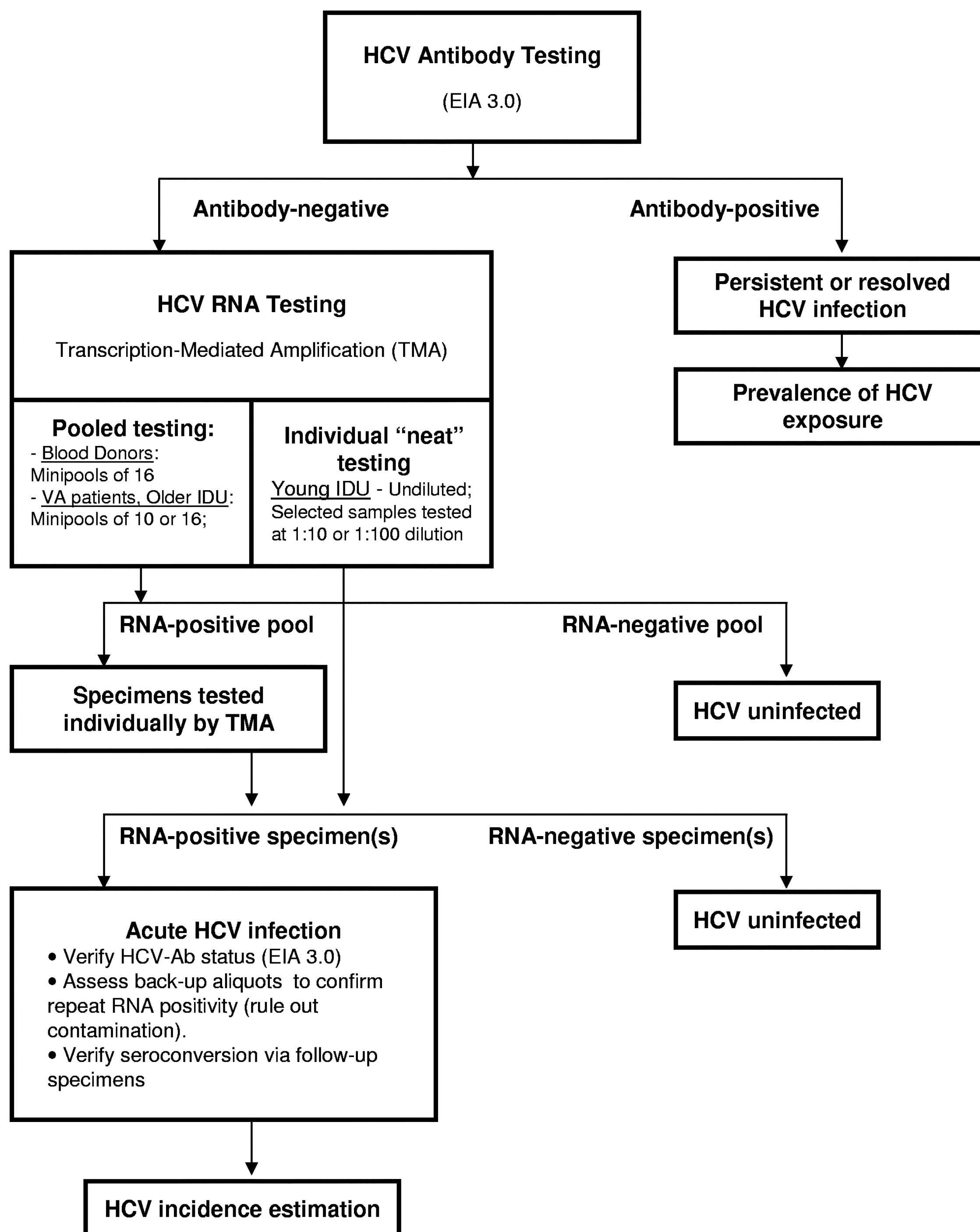

FIG. 1. Testing algorithm for screening of plasmas to identify individuals acutely infected with HCV (i.e., antibody-negative/RNA-positive specimens).

incidence rate (per 100 person-years), n_{vsn} is the number of viremic seronegative persons (i.e., acute infections) detected cross-sectionally, N_{sn} is the total number of HCV-seronegative individuals evaluated, and T is the estimated mean number of days between detectable viremia and seroconversion (i.e., the preseroconversion window period). In using this algorithm, we first evaluated the rate of detection of viremic seronegative infections (n_{vsn}/N_{sn}) for each population. Confidence bounds (95%) were computed by assuming that the number of acute infections (n_{vsn}) has a binomial distribution. The prevalence estimate was multiplied by 365 (days) and divided by the estimated duration, in days, of the viremic preseroconversion window period (T) to obtain an incidence estimate. Using Bonferroni inequality, conservative 95% confidence bounds for the incidence rate estimate were computed by combining the 99% CI on the estimated window period and the 95% CI on the prevalence rate (38).

To assess the accuracy of this testing strategy and incidence estimation, the projected HCV incidence rates derived from the cross-sectional data were compared with observed incidence data (based on seroconversion during longitudinal follow-up) for the various cohorts, where available (blood donors [47], young IDU [22, 43], and older IDU [34; Brian Edlin, unpublished data).

TABLE 2. HCV-viremic seronegative infections, estimated HCV incidence, and observed HCV incidence in sample populations

Population	No. of anti-HCV-negative individuals (N_{sn})	No. of anti-HCV-negative/ HCV RNA-positive individuals (n_{vsn})	Proportion (95% CI) of anti-HCV-negative/ HCV RNA-positive individuals (per 1,000)	Projected incidence rate (95% CI) (per 100 person-yr)	Observed incidence rate (95% CI) (per 100 person-yr)
Blood donors	4,620,687	29	0.0059 (0.0032, 0.0084)	0.0054 (0.0036, 0.0072)	0.0028 (0.0021, 0.0034)
VA patients	835	1	1.20 (0.03, 6.65)	0.86 (0.02, 7.1)	
Young IDU	618[a]	34	49.0 (32.8, 72.0)	39.8 (25.9, 53.7)	33.4 (28.0, 39.9)
Older IDU					
≥10 yr of exposure	72	4	55.6 (15.3, 136.2)	39.8 (9.1, 115.0)	15.4 (9.9, 22.9)
<10 yr of exposure	75	7	93.3 (38.4, 182.9)	66.9 (23.3, 153.6)	33.1 (23.1, 45.7)
Total	147	11	74.8 (37.9, 129.9)	53.7 (23.4, 108.8)	22.5 (17.2, 28.8)

[a] A total of 618 (90.5%) anti-HCV-negative samples, as tested by EIA 3, were available from 683 HCV-negative samples.

RESULTS

HCV seroprevalence. Confirmed HCV seroprevalence among 4,620,687 blood donations was 0.1% (Table 1). HCV seroprevalence among 790,989 first-time donations was 0.34%, and that among 3,629,698 repeat donations was 0.08%. Among IDU, seroprevalence varied based on the duration of HCV exposure (i.e., years of injecting) and was significantly higher for older IDU (1,425/1,601 persons [89%]) than for younger IDU (435/1,095 persons [40%]).

HCV incidence estimates. Table 2 presents the results of the RNA screening algorithm and detection of acute infection. The proportion of acute infections detected per 1,000 seronegative specimens roughly paralleled the prevalence rate for each study population. The mean estimated time from RNA positivity to EIA 3.0 positivity (window period), based on the analysis of serial samples from the 58 seronegative plasma donors, is shown in the survival curve in Fig. 2. The mean viremic preseroconversion window period (T), estimated to be 50.9 days (95% CI, 46.1 to 55.8 days), was used to project incidence in our algorithm. Incidence rates projected using the formula previously described ranged from 0.0054 per 100 person-years for blood donors to 53.7 per 100 person-years for older IDU. Among older IDU, rates ranged from 39.8 to 66.9 per 100 person-years, relative to the years of IDU exposure. Observed incidence rates were available for comparison to projected incidence data for three of the four study populations and are shown in the last column of Table 2.

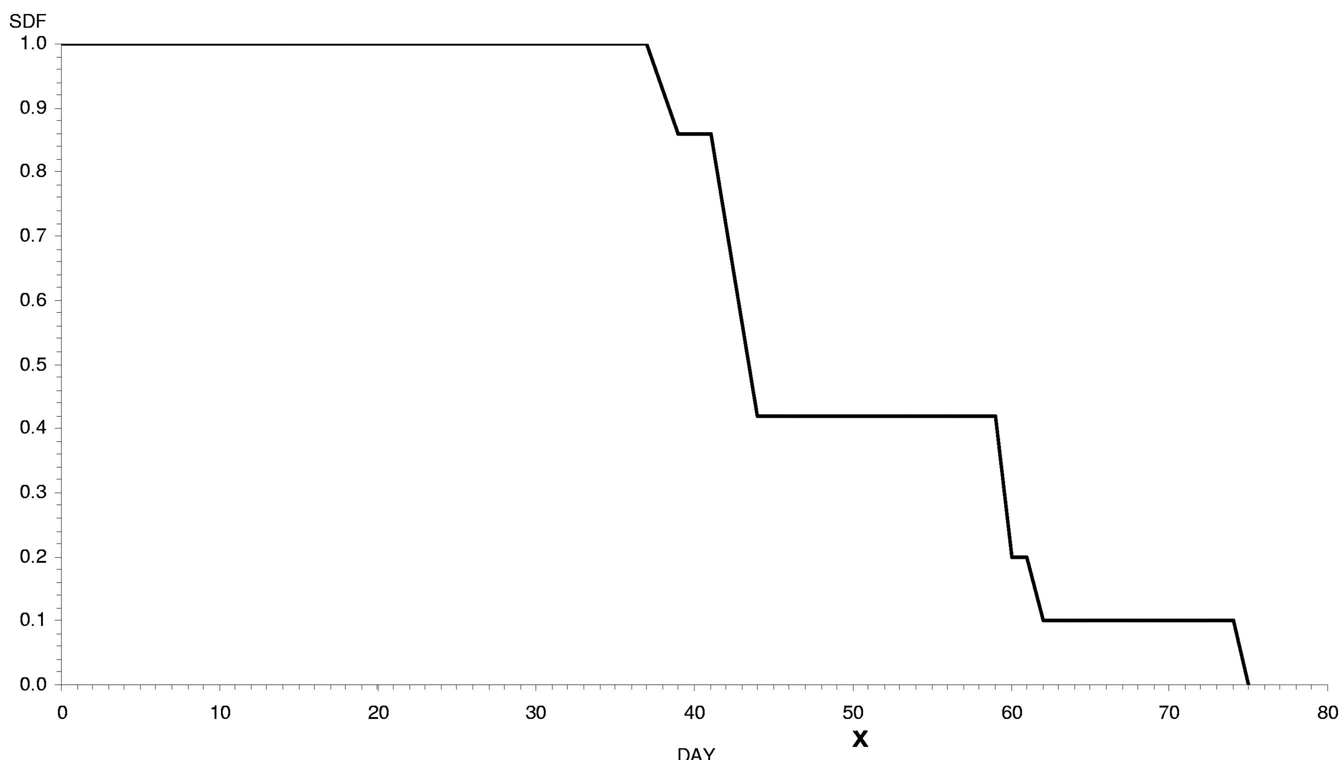

FIG. 2. Survival curve depicting the time from RNA positivity to EIA 3.0 positivity (window period) derived from 58 source plasma donors. X, the mean of 50.9 days.

TABLE 3. Comparison of HCV RNA testing results of dilution (pooled) strategy relative to individual testing strategy[a]

Pooled testing result at indicated dilution	No. (%) of HCV RNA-positive/anti-HCV-negative specimens detected using individual testing[b] (n = 28)	Median (range) no. of days to seroconversion (IQR)	Kruskal-Wallis P value
1:10			
RNA positive	25 (89.3)	53.0 (43.3–78.3)	1.0
RNA negative	3 (10.7)	45.5 (45.5–98.0)	
1:100			
RNA positive	19 (67.9)	47.8 (42.0–65.0)	0.07
RNA negative	9 (32.1)	77.0 (45.5–101.5)	

[a] Based on 28 RNA-positive, anti-HCV-negative specimens from screening and follow-up of young IDU cohort (see Materials and Methods) (23).
[b] Includes one individual who was confirmed to be HCV positive but who did not develop anti-HCV, possibly in association with HIV coinfection.

Evaluation of pooled sample testing compared to individual testing strategy for detection of HCV RNA during acute infection. Of 40 confirmed viremic seronegative samples detected by individual sample testing of the young IDU cohort, 28 had follow-up data confirming seroconversion (12 were lost to follow-up). Samples were tested at 1:10 and 1:100 dilutions to mimic the pooled sample testing strategy. At the 1:10 dilution, 25 of 28 (89.3%) individually confirmed infections were detected, whereas at the 1:100 dilution, 19 (67.9%) were detected (Table 3). Follow-up data were reviewed to assess the time to subsequent detection of anti-HCV. There were no differences in time to anti-HCV detection between HCV RNA-negative and RNA-positive samples tested at the 1:10 dilution, but there was a borderline difference (P = 0.07) in median times to observed seroconversion among those who tested HCV RNA negative (77 days) and those who tested positive (47.8 days) at the 1:100 dilution.

DISCUSSION

This study demonstrates the utility of an HCV RNA and anti-HCV testing algorithm to identify acute HCV infections from cross-sectional screening, as well as the use of this testing algorithm to estimate HCV incidence. For four populations with various risks of HCV, the proportion of detected viremic seronegative HCV infections, assumed to represent incident infections, increased in parallel, with the lowest proportion detected among blood donors and the highest detected among long-term IDU. The HCV incidence estimated from the number of detected viremic seronegative infections was most accurate among young IDU, for whom the estimated incidence was 39.8%, comparable to the observed incidence of 33.4%. Younger IDU are among those at highest risk of HCV infection (21, 23, 52) and, not surprisingly, demonstrated the highest rate of HCV infection of all groups included here. The observed HCV incidence in blood donors and in older drug users was lower than that projected from cross-sectional samples; however, confidence intervals for projected and observed incidence overlapped across all groups. Among the blood donors, the discrepancy between projected and observed incidence was likely attributable to donor status and possibly to differences in incidence in different demographic strata (53). HCV window-phase infections are known to be three times more likely to be detected in first-time donors than in repeat donors (47).

Various factors may contribute to discrepancy between projected and observed rates of HCV infection, including the testing interval and follow-up rates within groups. Overestimation of incidence may result if some viremic seronegative infections are misclassified as acute infections. Such "immunosilent" infections have been documented for immunocompromised hosts who are incapable of generating a detectable level of HCV-specific antibody and, rarely, for immunocompetent hosts (41). Viral factors may also contribute to delayed antibody responses in rare circumstances, as recently documented by our group (5). Variability in HCV RNA detection and natural history during early infection may also result in differences between projected and observed incidence rates for these groups, as discussed below in more detail.

Based on systematic HCV NAT screening of blood donors, immunosilent infections appear to be rare; the vast majority (>99.9%) of HCV-viremic donors are detected by antibody screening (46), and only 3 (4.5%) of 67 RNA-positive/antibody-negative donors who enrolled in a prospective follow-up study conducted following implementation of NAT screening failed to seroconvert by EIA 3 within 1 year of RNA detection (47). It is noteworthy that these three immunosilent cases were detected during the first year of donor NAT screening, with no confirmed immunosilent cases detected over the subsequent 6 years of screening of over 12 million U.S. whole-blood donations annually, indicating that persistent viremia in the absence of seroconversion is a very rare phenomenon in immunocompetent populations (S. Stramer, personal communication). Transient infections have also been documented among the young IDU population, although they appear to be rare, with two confirmed events among 121 infections detected over a 6-year period (K. Page-Shafer, unpublished data). Intercalations of HCV RNA positivity which may occur in the early natural history of HCV infection (40) may also result in a small proportion of participants being misclassified as cleared or uninfected and may result in differences between projected and observed incidence rates.

Regarding human immunodeficiency virus (HIV) infection, none was detected among the blood donor group members, who were tested concurrently. VA patients were not tested for HIV in this study, although a recent study found 8.4% HIV prevalence among VA patients with chronic HCV infection (6). Among the older IDU group members, HIV has been documented overall at 11.9% (51), and among the young IDU tested for this paper, 881 were tested for HIV and 3.1% were HIV infected. Delayed antibody responses have been documented for HCV-viremic individuals, including those coinfected with HIV, in several settings (9, 15, 44), including drug users (4, 42). Older drug users and those with the greatest number of years of injection exposure have a much higher HCV prevalence (34, 48), and many of these infections may have occurred 20 or more years previously. Coinfection with HIV is more likely in this older group (30), possibly resulting in impaired antibody responses to acute HCV infection or HCV EIA seroreversion after prolonged HCV infection as HIV-induced immunosuppression evolves. Although the num-

ber can be expected to be low, aberrant antibody responses associated with HIV in these populations and the potential misclassification of acute infection may result in overestimation of the projected HCV incidence rates, especially compared to the observed incidence based on anti-HCV detection methods. Since 5% or more of HCV/HIV-coinfected patients may be anti-HCV negative and potentially misclassified as acutely infected (7, 12, 17, 32), caution is advised in the use of our proposed testing strategy to estimate incidence in HIV-infected or other immunosuppressed populations.

We acknowledge other potential limitations of this testing algorithm as well. First, the 50.9-day mean length of the viremic preseroconversion window period used in this testing algorithm was derived from a cohort of acutely infected plasma donors. This sample group is comprised of individuals with community-acquired HCV infection, generally assumed to be IDU (20), and hence should be relevant to the populations represented in our analyses. Other estimates of this window period differ somewhat: Glynn et al. (20) reported a mean of 56.3 days (95% CI, 44.8 to 67.8 days) for plasma donors, and Busch reported a mean of 60 days for the blood transfusion recipient setting (9). It is possible that in addition to the infection route, other factors, including the amount or size of the inoculum (39), exposure frequency, and even demographic factors, could influence the natural history of acute infection and hence the duration of the viremic preseroconversion phase. Differing distributions of viral genotypes within these populations could potentially influence window period estimates as well. Viral genotypes are known to influence disease outcome and the response to treatment (24), and although to date there is little evidence of variation in acute-phase HCV by genotype (39), data are limited, and further studies are warranted to assess window period estimates by viral subtype.

NAT testing for HCV RNA and other blood-borne viruses is now regularly and effectively used in the blood, plasma, and organ donor screening settings to reduce the residual risk of transfusion- and transplant-transmitted infections (47). In donor screening, pooled sample testing, as was done for most of the groups assessed here, is now routinely employed for HCV, HIV, HBV, and West Nile virus NAT. Among such low-prevalence/incidence populations, this approach is the most viable in terms of testing capacity, turn-around time, and cost-effectiveness. Among populations with a high prevalence and incidence of HCV, such as IDU, pooled testing may be less efficient. First, a large proportion of anti-HCV-negative samples can be expected to be reactive, leading to the majority of pooled tests having to be retested and resolved individually. Second, in high-incidence populations, such as young IDU, a small but significant proportion (10% at a 1:10 dilution and 32% at a 1:100 dilution) of window-phase infections may be missed using the pooled sample approach. The HCV inoculum size may vary by route of infection, possibly affecting viral load during the window phase such that pooled or diluted sample approaches may be less effective at detecting viremia in early infection. Community-acquired infection is believed to be associated with a differing immune response as well (29). Further studies of viral kinetics and immune responses during early HCV infection following different exposure routes are needed to address these issues.

Aarons et al. (1) used a similar approach to detect acute HCV infections among IDU in London. Seronegative samples were tested retrospectively for HCV RNA, using minipools of 20 samples each; positive tests were resolved using individual dilution (1:20) testing. HCV viremic seronegative infections were identified, and incidence was estimated based on a 58-day window period (9). The estimated incidence from cross-sectional testing (12.5%) was compared to the observed incidence (16.1%; $n = 2$) among IDU who were tested multiple times during the same time period. The authors noted that poor storage and the dilution factor may have contributed to the underestimation of estimated incidence. Our results showing that 10% to 30% of acute infections may go undetected upon dilution support this possibility. In both the above-mentioned study and this one, 5% to 7% of anti-HCV-negative IDU were confirmed to have HCV infection. All of these infections would have been missed by conventional serological screening, and many would have been missed by commercially available PCR-based quantitative HCV viremia assays, due to the relatively low sensitivities of these tests. Together, these results support the use of sensitive HCV RNA screening of high-risk populations.

In conclusion, the utility of pooled NAT screening to detect window-phase infections is well recognized for blood supply safety. With respect to screening for acute HCV infection in high-risk populations, individual screening will provide the best estimates of HCV incidence and can be an effective tool for public health surveillance and case-finding purposes. On an individual level, identifying acute HCV infection may help in reducing transmission risk from acutely infected individuals, who may be more infectious due to high-titer viremia, as seen with HIV (54). Since it is now recognized that treatment of acute HCV infection is highly efficacious (26, 28), this testing approach may be used to inform and improve health care needs of IDU. Finally, this testing strategy will be highly useful for identifying high-incidence populations for future intervention studies, such as preventive HCV vaccine trials.

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At the time of collection and analyses of these data, we had no commercial or other association that might pose a conflict of interest with this research. B. H. Phelps was an employee of Chiron Corporation, which performed the HCV RNA testing; he did not have any financial interest in the conduct of the research. M. P. Busch is on the scientific advisory board of Gen-Probe.

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