Personal risk networks and the molecular epidemiology of Hepatitis C amongst injection drug users in Winnipeg

by

Lena Shah

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

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Department of Community Health Sciences

University of Manitoba

Winnipeg

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Abstract

Introduction: Despite prevention programs, HCV is still on the rise within the injection drug use population (IDU). In this, Phase II of the Winnipeg Social Network Injection Drug Use study (Winnipeg SNS IDU), individual data and social network data as well as molecular data were used to examine transmission of hepatitis C.

Methods: A public health nurse collected interview data and blood samples from 435 consenting individuals in Winnipeg, who used drugs intravenously during the 6 months preceding the interview date. Blood samples were tested for the presence of antibodies against HCV, and positive specimens were genotyped. Logistic regression analyses were used to correlate data with HCV genotype. Association indices were used to determine the degree of segregation of sequences between study participants, based on key characteristics.

Results: Prevalence of HCV in the study population was 54.4%. The genotypes circulating in this population were 1a (82, 59.0%), 3a (47, 33.8%), 2a (3, 2.2%), 2b (2, 1.4%), 1b (5, 3.6%). At the multivariate level, HCV genotype 3a was associated with younger age and injecting on the street, when compared to genotype 1a. Moderate segregation of sequences was seen for those individuals who had injected in hotels and/or public washrooms, had moved to Winnipeg in the past year and/or had participated in the sextrade.

Conclusions: The findings suggest distinct networks of HCV transmission exist in the study population, such that more intra-network connectivity than inter-network connectivity is present. Targeted prevention and treatment strategies should be used in the local IDU population, as some public health messages may not readily diffuse to all groups.

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First and foremost, I am indebted to Dr. John Wylie for his guidance throughout my Master's thesis. Dr. Wylie tirelessly guided me through many details of my thesis and his patience has been much appreciated. This document would not have been possible without his assistance.

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On a personal note, thanks to Rena Pandya, who always takes my mind off the mundane and who understands me better than anyone and to Mohyna Ragoonaden, thank you for being with me through every painstaking step of the last years (we will always have Fuel Café). My heartfelt thanks to Sunayna Gupta, my little princess, who literally supplied me with all my ridiculous requests (yeah, bring my medium double double and don't be late). Finally, to Mum and Papa and my sister Reena, for giving me every opportunity to pursue my passions both in and out of school and always reminding me of my life outside of work.

Dedication

To my grandmother and great grandmother - Hasumati Shah and Hirakunwar Shah

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Chapter 1: Introduction

1.1 Burden of disease

Drug injection is the most important transmission route of Hepatitis C (HCV) in Canada [1]. Health Canada estimates that 63% of new HCV infections are related to injection drug use each year [2]. Of new injection drug users (IDU), it is estimated that greater than half will become HCV positive within 6-12 months [3]. Studies published by the Hepatitis C Prevention, Support and Research Program of Health Canada suspect that injection drug use is on the rise, but currently statistics on IDU and those infected with HCV are limited to estimates. Prevalence of HCV amongst IDU in Winnipeg has not been determined, but in Manitoba, age-standardized rates designate Winnipeg as having the highest observed rate of HCV in the general population [4].

Clinical progression of HCV includes liver cirrhosis, fibrosis, chronic liver disease and hepatocellular carcinoma. In Canada, the economic burden of HCV is estimated at $500 million dollars and projected to cost $1 billion dollars to the Canadian health care system by 2010 [5]. Currently, a vaccine for HCV does not exist.

The most important risk behavior involved in HCV transmission is sharing contaminated needles and borrowing of other used injection equipment [6-10]. Current recommended prevention strategies for HCV amongst IDU include harm reduction strategies, which involve providing safe injection equipment to IDU [2]. Although "needle sharing" has decreased, HCV incidence rates continue to be high and certain studies have shown that availability of clean needles is not adequate to combat the complex social determinants of addiction and related risk activities [6, 11-13].

To explore potential strategies directed specifically at HCV prevention, better understanding of its transmission through high risk populations is needed. In a larger sense, social network patterns may elucidate movement of this pathogen through an already high risk population. In addition, movement of this virus through the IDU population may be described using molecular epidemiology.

1.2 Social Network approach

The social network approach, in the context of infectious disease research, seeks to understand how individuals interact with each other and how those interactions contribute to an elevated or decreased risk for disease acquisition. A social network is defined as a set of interconnected nodes, (i.e. people), with the links between the nodes representing social relationships (Figure 1) [14, 15]. Sharing needles involves personal interactions within a group and is one of the types of connection that links people to each other in a social network. Use of social network analysis to examine the spread of infectious disease is an innovative approach to investigating pathogens [16-18]. Social network analysis was the key technique which characterized the method of transmission at the beginning of the HIV epidemic in the 1980's [18]. Investigating interactions in a group is particularly useful for blood-borne pathogens, such as HIV and HCV, because their transmission involves close personal contact between group members. This type of contact can be more readily recalled, as opposed to the casual contact associated with transmission of respiratory infections.

Studying social networks illustrates the pattern of spread of a pathogen through a group and can outline key factors influencing the manner of its spread. In the sociocentric approach, the network is investigated as a whole and focuses on the pattern

of connections in the entire network. In the egocentric approach, the network is centered on one person and deals only with his or her links to other people (Figure 2).

Egocentric or personal networks of injection drug users, in the context of HCV, reflect individuals' potential as intermediates of transmission within a population. They can also help characterize risk factors for disease acquisition. In New York, Neaigus *et al.* (1996) found that among those who share syringes, having a high-frequency injector or a network member greater or equal to ten years older than them in a personal risk network increased the probability of being HIV infected [19]. The results of Neaigus *et al.* (1996) suggest that analysis of personal networks is useful for understanding the transmission of blood-borne pathogens and that the risk of disease acquisition via sharing needles and other injecting equipment may be mediated by an individual's set of contacts.

Figure 1: A diagram of a social network [15]. Dots represent individuals and the lines linking them are a social interaction, such as sexual relationship.

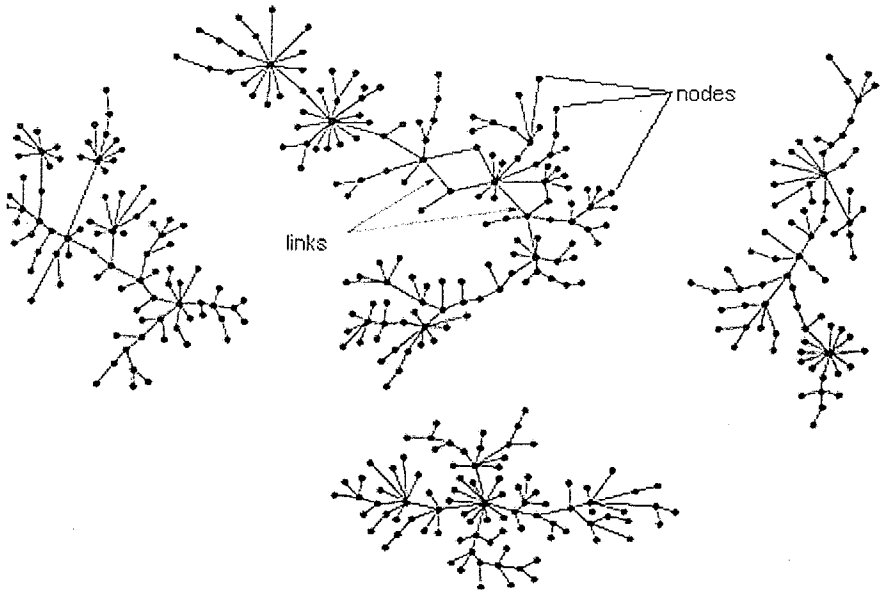
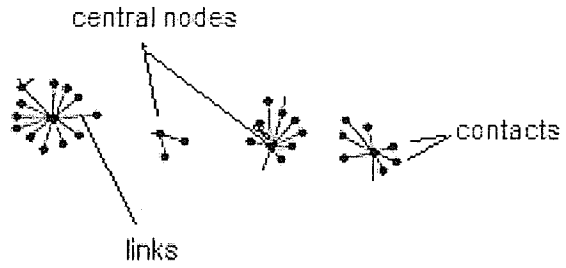


Figure 2: A diagram of egocentric networks extracted from Figure 1 [15]. Networks are built upon a single central node (i.e. individual) and studied independently from one another.



1.3 Molecular epidemiology

Molecular epidemiology, the study of biomarkers, is useful for assessing HCV genotype distribution and its transmission [20, 21]. Molecular epidemiology has been used to expand knowledge about the evolution of infectious diseases, but also serves as a basis to control their spread [22].

From a molecular perspective, six known genotypes (genetic sequences), designated 1 through 6, and several other subtypes have been identified for HCV [21, 23]. Genotypes of HCV differ in their pathogenesis and responsiveness to treatment [24]. According to the Hepatitis C Prevention, Support and Research Program, difficulty in stemming the epidemic of HCV among IDU may in part be due to the number of HCV genotypes circulating within this population [25].

Evidence that some genotypes (1a and 3a) are specifically associated with IDU populations exists [21, 26-28] . In Canada, less is known about genotypes circulating among IDU. Previously, a study in Montreal found genotype 1 representing 60% of HCV among blood donors and patients (genotype 1a and 1b each represented 30.2% of the study population) [29]. Detailed analysis of genotype data specifically amongst IDU in Canada is not currently available.

However, the molecular epidemiology of HCV within the social networks of IDU has not been thoroughly investigated. In the Czech Republic, Krekulova *et al.* (2001) found predominance of genotype 1a among heroin users and genotype 1b among amphetamine users [30]. In Australia, an analysis of HCV, Hepatitis B and Human immunodeficiency virus (HIV) by Crofts *et al.* (1999), found a significant association

between drug choice and disease outcome; heroin injectors were at greater risk of HCV infection whereas amphetamine injectors were at greater risk of HIV infection [31].

The results of Crofts *et al.* (1999) and Krekulova *et al.* (2001) suggest that smaller distinct transmission networks exist within the larger injection drug user population and that the characterization of HCV genotypes circulating in a given population can potentially identify these transmission networks [30, 32]. The distinct networks may separate along social lines and have differing risks for acquisition of blood-borne pathogens such as HCV.

1.4 Purpose of study

Current research efforts and prevention strategies primarily target individual risk behaviors and have focused on HCV prevalence or social determinants [12]. The present study will be the first to link social network analysis and molecular genotyping to study blood-borne pathogens in Canada. The intention of this study is to provide information on HCV transmission patterns among IDU. The incorporation of social and molecular data may identify whether distinct transmission networks for HCV exist within the larger IDU population in Winnipeg. Transmission networks are social networks within which a given pathogen is spreading via the social risk behaviour connection between individuals [33]. From a molecular perspective, phylogenetically related HCV sequences would be identifiable (and expected) within a given transmission network. In this study, the transmission network may also be identifiable by social behaviours; this pattern will be evident through correlations between specific genotypes and specific social variables (e.g. drug type injected).

Currently data on HCV genotype distribution within the IDU population of Winnipeg is not available. The proposed study will inform the research community of diversity of HCV genotypes amongst IDU in Winnipeg. The integration of social network analysis with molecular data will provide information to better understand HCV transmission amongst IDU. This analysis has the potential to reveal approaches to reduce or prevent HCV spread by identifying key risk behaviours or demographic characteristics associated with transmission at the molecular-level within the larger IDU population.

The study described in this thesis is a part of a larger CIHR funded project. The five specific aims of the entire project are (from Research Proposal of John L. Wylie):

1. Analyze the molecular epidemiology of HCV within social networks.
2. Correlate seroprevalence of HCV and HIV with social network variables.
3. Analyze the social context of syringe sharing amongst IDU.
4. Correlate immune system status with social behavioral data.
5. Construct and analyze sociometric networks of IDU.

For this thesis, I have undertaken analyses incorporating Aim 1 and part of Aim 2 above. The specific objectives for my thesis have been described below:

Objective 1: Correlate the seroprevalence of HCV with individual and social network variables.

Objective 2: Determine which sociodemographic and sociobehavioural variables are correlated with infection by either HCV genotypes 1a or 3a.

Objective 3: Determine whether correlations between social variables and HCV subtypes (i.e. below the genotype level) exist.

The latter two objectives are designed to use molecular data to identify transmission networks within the larger IDU population. Objective 2 uses genotype assigned based on sequence data whereas Objective 3 examines variation within genotypes and uses all of the sequence data generated.

The overall hypothesis guiding my research is that networks of IDU, segregated along socio-economic or behavioural lines, can be identified using genotype data. Objective 1 focuses on associations of individual and personal risk network behaviours of IDU and HCV status (positive and negative) and was included to provide a broader understanding of HCV amongst IDU in the Winnipeg population, prior to focusing on the comparison between molecular types and subtypes. The focus will be on genotypes 1a and 3a as these genotypes have been associated with, and are most prevalent amongst IDU.

The primary investigators of the overall study are Dr. John Wylie of the Cadham Provincial Public Health Laboratory (CPL) with expertise in medical microbiology and Dr. Ann Jolly, an epidemiologist at the Centre for Infectious Disease Prevention and Control, Health Canada, Ottawa and the University of Ottawa. As a graduate student at the Master's level, I conducted the necessary molecular techniques required for genotyping HCV from serum specimens, with the assistance of a technician. With a background in undergraduate Biology and graduate coursework in Community Health Sciences (Epidemiology and Biostatistics), I was able to investigate Objectives 1-3, as described, to fulfill requirements of my thesis project.

The following chapters will review the literature, discuss the methodology used, display the results, and discuss findings generated from all analyses.

Chapter 2: Literature Review

This review of the literature will discuss significant studies involving injection drug users in Winnipeg, social network analysis relating to the research of transmission, risk factors for HCV, molecular studies of HCV, and finally the importance of combining molecular and social network data in the study of blood borne pathogens such as HCV.

2.1 Winnipeg Injection Drug Users

The most notable studies with high-risk populations relating to bloodborne pathogens in Winnipeg, have been the Winnipeg Injection Drug Epidemiology Study (WIDE) and a study of viral hepatitis amongst street-populations [34, 35]. The first, WIDE, focused on human immunodeficiency virus (HIV) correlated with individual risk behaviors. The second was a study documenting Hepatitis A (HAV), B (HBV) and C (HCV) among street-involved individuals.

WIDE was created as a cross-sectional survey of injection drug users in Winnipeg and documented intravenous drug use activities, prevalence of HIV, and risk factors associated with infection. WIDE showed an elevated risk of HIV transmission amongst IDU and that the predominance of IDU as an exposure category for HIV was increasing [34]. An increase of HIV infection prevalence among Winnipeg IDU from 2.3% during the period 1986-1990 to approximately 12.6% in 1998 was demonstrated. After multivariate analysis, WIDE found HIV positivity to be associated with age groups 25-29 and 30-34, sharing needles, injecting cocaine, being involved in sex trade, and men having sex with other men [34].

The recommendations from WIDE were specific to injection drug use and HIV transmission and included preventing injection drug addiction, in addition to harm reduction. Although WIDE did not study HCV seroprevalence, it recommended multi-sectoral strategies be developed and implemented to prevent further extensive transmission of HIV and other blood borne pathogens, such as HCV, among IDU.

However, in an outbreak of HIV in Vancouver (2001), harm reduction initiatives proved sufficient to reduce HIV transmission significantly, but insufficient to reduce HCV transmission in an IDU population [36]. Patrick *et al.* (2001) suggested high transmissibility of HCV among those injecting frequently and using cocaine were responsible for the difficulty in controlling HCV spread [37]. Results of the WIDE study and a separate national drug use study by Poulin *et al.*(1998) showed a shift in predominant drug choice from Talwin and Ritalin (a mixture of the prescription drugs Talwin® and Ritalin®, also known as Ts and Rs) to cocaine use as an emerging problem in Winnipeg [38]. The reason cocaine use is considered a greater hazard for HCV transmission is because it involves greater frequency of injections than Talwin and Ritalin.

WIDE cited an outbreak of HBV and HCV among Winnipeg street-involved youth, many of whom were injection drug users in 1995. Since then, Moses *et al.* (2002) conducted a study of viral hepatitis in a Canadian Street-involved population [35]. Canadian studies involving HCV have focused on its determinants and have served primarily to document prevalence amongst high risk groups such as street-involved people which include, but do not focus on injection drug users [35]. Literature leading to this study suggested higher HCV infections amongst street involved individuals and this

study's objective was to further explore risks of transmission. The Canadian Street-involved study revealed that injection drug use, sex trade work and age over 25 years are significantly associated with HCV after multivariate analysis [35].

Both WIDE and the Canadian Street-involved population studies captured relevant information regarding prevalence of HIV and HCV amongst injection drug users. According to these two studies, conducted in the same city, potential key risk variables for transmission of these viruses include being involved in sex trade, age over 25 years, and injection drug use. However, neither study fully investigated the dynamics of HCV transmission within IDU populations and the potential requirement of specialized preventive interventions for HCV.

2.2 Social Network Analysis

Literature suggests that the complex determinants of risk factors such as needle-sharing are embedded in the social context of how people interact in a group [6, 12]. Few studies have looked at this aspect of drug injector's lives; therefore a justifiable need exists to correlate seroprevalence of HCV and social network variables.

Research using social network analysis to study infectious disease has been conducted, but literature on this topic is relatively limited. Although, clearly important, the social context of syringe sharing is rarely contemplated and rarely studied. Most studies have been related to HIV transmission. Social network methods have been used to explore and understand the HIV/AIDS epidemic; initially to identify the sexual transmission of HIV/AIDS and later, spread of HIV/AIDS amongst IDU sharing needles [39, 40].

Sharing needles is a key risk behavior for HIV and HCV [1, 19]. However the risk of HIV infection among new injectors who share syringes appears dependent on whether their personal risk networks would be considered high risk (e.g., having contact with someone who injects on a daily basis) [12]. Neaigus *et al.* (1996) found that among those who share syringes, associating with a high-frequency injector or an older individual ($\geq$10 years older) increased the probability of being HIV infected [19]. In this case, sharing needles was not the key risk behavior associated with disease transmission but rather sharing needles with individuals who have a high likelihood of being infected with HIV. This type of study has not yet been conducted for HCV.

2.3 Molecular epidemiology

2.3.1 HCV Genome and Genotypes

HCV is an RNA virus related to the *Flavivirus* family. The 9.6 kb genome is a single-stranded linear RNA of positive sense containing an open reading frame (ORF) flanked by a 5' and a 3' untranslated region (UTR) (Figure 3). The ORF encodes a large polyprotein precursor that is subsequently cleaved into various structural and nonstructural proteins. The structural proteins include the capsid or core protein (C), two envelope proteins (E1 and E2), and a smaller protein (P7). The most variable regions of the HCV genome, including the hypervariable regions (HVR1 and HVR2), are found in genes encoding the envelope proteins E1 and E2 (Figure 3).

Currently, six known clades or types of HCV and several subtypes have been identified. Based on genetic differences, HCV strains can be classified into distinct genotypes, numbered 1 through 6 [23]. Within each genotype, more closely related subtypes are designated with lower case letters, eg. 1a, 1b, 1c. The determination of

genotype and subtype is based on phylogenetic analyses of full or partial sequences of HCV strains. The clades differ by greater than 30% whereas the subtypes differ from each other by less than 30% (Table 1). HCV is variable and has the potential to form quasispecies (closely related mutations from the original virus) during replication [42]. Quasispecies can differ by 1-5% of their nucleotide sequence in individuals (Table 1).

Methods for determining genotypes include restriction fragment length polymorphism (RFLP) assays, hybridization assays, PCR with type-specific primers and sequencing-based methods [43]. Studies comparing sensitivity and specificity of these techniques have been published [44, 45]. Although more costly and labour intensive, the sequencing-based method is considered the most accurate and detailed method for HCV genotyping [43].

2.3.2 Epidemiology of HCV Genotypes

At the global level, HCV genotypes are distributed non-randomly. Typically, genotypes 1, 2 and 3 are found in Europe and North America and the Far East, genotype 4 in the Middle East and Africa, genotype 5 in South Africa and genotype 6 in Southeast Asia [46, 47]. Subtypes 1a, 1b, 2a, 2b, 2c, and 3a are responsible for over 90% of infections in North and South America, Europe, and Japan, although the distributions of these subtypes vary from country to country [42].

Internationally, molecular studies have focused on geographic clustering, pathogenicity, infectivity and response to antiviral therapy based on genotype [48, 49]. For example, response to therapy correlates with genotype. People with genotypes 2 or 3 have a higher sustained response rate (60-70%) to combination therapy than genotype 1 (20-30%) [5]. The duration of treatment is also influenced by genotype; previously

untreated patients with genotype 1 double their chance of a sustained response when treated for 12 months instead of 6 months, while 12 month treatment for patients with genotypes 2 or 3 does not improve response rates over 6 month treatment [43]. However, other factors such as stage of fibrosis or cirrhosis, viral load, age, duration of disease and excessive alcohol consumption also influence pathogenesis of HCV and therefore response to therapy [50].

Phylogenies, diagrams of branching patterns representing the estimated evolutionary histories among species of animals, plants or viruses, are also tools used to study molecular epidemiology [51]. Phylogenetic analysis of HCV has concentrated on geographic clustering, for example, to determine relatedness of HCV genotypes between different countries [27, 52, 53]. Migration of individuals has introduced the potential for strains to cross geographic boundaries, and an ongoing need to monitor genotypic diversity within populations has been noted in the literature [29]. In Canada, fewer studies of the genotypes of HCV in circulation have been published. Of those that are published, the focus has largely been to compare typing techniques including PCR by type-specific primers and hybridization assay [45, 54]. These have also focused on nosocomial/clinical samples including recipients of blood transfusions and hemophiliacs. In addition, the majority of clinical studies focus on the genotype, and not the subtypes and the variety of subtypes within the population.

Figure 3: HCV genome [41]

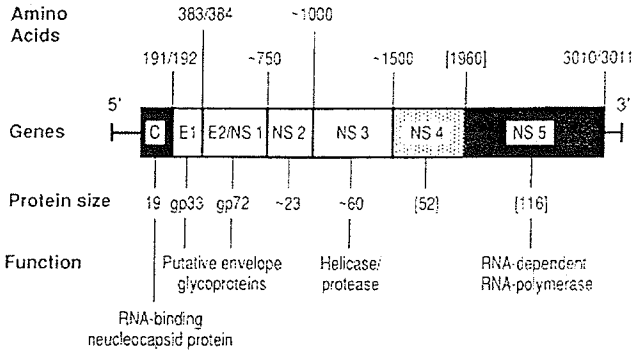


Table 1: Genetic Heterogeneity over the entire HCV Genome [42]

Hierarchic Strata	Sequence Variation, %
Genotype (1-6)	**30-50**
Subgenotype (subtype)	**15-30**
Quasispecies	**1-5**

2.4 Molecular epidemiology of HCV and social networks in IDU

2.4.1 Studies of HCV combining molecular and social data for IDU

Studies to date suggest the distribution of HCV genotypes within the IDU population may differ between drug choice and other variables such as education [30]. In the Czech Republic, Krekulova *et al.*(2001) found predominance of genotype 1a among heroin users and genotype 1b among amphetamine users [30]. In Uzbekistan, Kurbanov *et al.* (2003), showed age was also considered to be an independent determinant of HCV genotype amongst IDU [27]. A pilot study conducted in Winnipeg (Winnipeg Social Network Injection Drug User Study) showed a significant association with genotype 3a and post-secondary education (unpublished results). The above findings suggest that distinct HCV transmission networks can be identified within the context of a larger social network. These distinct networks can potentially be linked to specific risk factors.

A group led by Campbell Aitken at Burnet Institute in Melbourne, Australia is the first to publish findings on the molecular epidemiology and social networks of HCV of injection drug users [55]. The Melbourne study consisted of interviewing and collecting blood samples from 199 IDUs. The recruitment involved interviewing index (seed) cases who described members of their injecting network and referred or introduced the contacts in their network for interview and blood sampling; the process was repeated with their network members in turn, creating a complex map of IDU relationships (sociocentric network). Molecular epidemiology techniques allowed identification of related HCV infections.

Aitken *et al.* (2005) found a statistically significant relationship between the social and genetic distances separating IDUs [55]. Although their results show that the

further separated individuals are socially, the less close are their genotypes, the confidence intervals were considered wide and the relationship was not very strong. Also, their regression analysis consisted only of social distance and genetic distance and and not other risk behaviours and demographic behaviours.

Transmission networks have been studied more thoroughly by comparing the phylogenetic analysis of HCV between IDU groups. Cochrane *et al.* (2002) and Van Asten *et al.* (2004) have analyzed the molecular epidemiology of HCV to understand transmission between geographic locations [28, 52]. The extent of sequence segregation between groups, called Association Indices (AI) (see below, section 2.4) were calculated to assess whether transmission of HCV spread through similar networks. The technique and software (Simmonics Sequence Editor version 1.3 [56]) used for the AI analysis was developed by Peter Simmonds, who has published extensively on the genetic analysis of HCV [48, 57-63].

Initially, Wang *et al.* (2001) published analysis using AI in their study of the relatedness of HIV variants infecting microglia and tissue macrophages outside the central nervous system [64]. Since then, Cochrane *et al.* (2002) used AI analysis to assess the extent of HCV transmission between 5 IDU communities in distinct Western European cities [28]. The authors showed that cities geographically isolated from one another had varying degrees of HCV sequence isolation, but that HCV populations were not completely isolated from one another. Finally, van Asten *et al.* (2004) compared clustering of HCV sequences and HIV sequences using AI analysis to describe spread amongst co-infected IDU. The latter study found that country-specific clustering

occurred with HIV, but HCV did not show clear phylogenetic clustering by geographic region [52].

To my knowledge, no other studies linking molecular transmission and social data of HCV amongst IDU have been published.

2.4.2 Association Indices

Historically, AI analysis was used for measuring the strength of association between animal and plant species, and later to measure social affiliations [65]. The AI is a quantitative measure of the amount of deviation of the number of species that occur together, from the number expected by chance [66].

As mentioned in section 2.4.1, AI analysis has recently been used to examine HCV transmission amongst IDU to compare geographic patterns of viral exchange. Peter Simmonds developed software that looks specifically at incompatibilities between the phylogenetic groupings of the sequences with the individuals' membership in specific groups (Simmonics manual). In the past, the software was used to correlate HIV and HCV sequence data to geographic location (i.e. between cities) [28, 52]. However, to date, other social variables, such as injection sites and factors affecting IDU behaviours, have not been correlated with HCV viral sequences using AI analysis.

For calculation of the AI, two sets of phylogenetic trees are constructed, termed the "observed" and the "control" trees. The "observed" phylogenetic trees are used to measure phylogenetic segregation between individuals, whereas the "control" trees determine how often this segregation would occur by chance. The "control" trees are created by random reassignment of the test sequences along the length of the "observed" tree. An association value for the "observed" tree is calculated by summation of values

individually calculated in each successive bifurcating node of a phylogenetic tree (i.e. a mathematical formula is used to calculate the degree of phylogenetic mixing). The AI is the ratio of the median of the latter value against the median association value calculated using the same technique for the control phylogenetic trees. The AI value takes a range between zero and approximately one, where values approaching zero represent almost complete segregation, and values greater than or equal to one, suggest no more segregation than would be expected by chance [28, 52, 64]. The use of bootstrap-resampled trees ensures that the robustness of the phylogenetic tree is represented in the data [52].

In comparison to the AI, other statistical analysis cannot simultaneously address the relative association between HCV subtypes and their correlates with social variables. Logistic regression can analyze correlates with categorical (bivariate) outcomes, such as HCV status or genotype, but cannot investigate the outcome of numerous HCV subtypes and the degree of sequence segregation. Alternatively, cluster analysis can be used to group data and measure degree of association between individuals (i.e. arrange data into clusters), but it cannot account for these clusters in the context of social data outcomes. In addition, neither logistic regression nor cluster analysis, have functions to compute phylogenies or tree association values to examine HCV subtypes.

A limitation to the AI analysis is that it considers the relative position of sequences to the nodes (proximity to one another), and does not account for branch length (viral divergence) and therefore, time of transmission [67]. Nonetheless, AI analysis is still the most thorough method of examining genetic segregation and correlating this variance with sociodemographic and sociobehavioural data.

2.4.3 Support for combining social and molecular methodology in the study of STD and blood-borne pathogens

Combining social data and molecular data is a recent development in STD and blood-borne pathogens [16]. In particular, combining social networks and molecular genotyping has been useful to study *Chlamydia trachomatis*, *Neisseria gonorrheae*, *Mycobacterium tuberculosis* and HIV [17, 68-70].

The decision to combine molecular and social network data is supported in the literature by Day *et al.* (1998) and Hesse *et al.* (1995) [16, 71]. Based on this literature, Drs. Wylie and Jolly collaborated on the Winnipeg IDU Social Network Pilot Study and on analysis of the sexual network patterns and molecular epidemiology of the sexually transmitted bacterial pathogens, *Chlamydia trachomatis* and *Neisseria gonorrheae*, in Manitoba [17, 69, 70]. Their results showed a great diversity of *Chlamydia* genotypes in the province and found genotypic diversity matched epidemiological data. The result was confirmation that contact tracing was accurate with respect to tracing the transmission routes of individual *Chlyamydia* types. The importance of this work was highlighted in an editorial by Dr. Alden S. Klovdahl supporting the use of sexual network analysis as a technique for 'effective disease control' [72].

Based on this literature review, a significant need for a larger scale molecular epidemiologic study of HCV exists. In addition, incorporating social network analysis, specifically personal risk network information, of IDU will expand understanding of HCV transmission within IDU populations.

Chapter 3: Materials and Methods

3.1 Study design

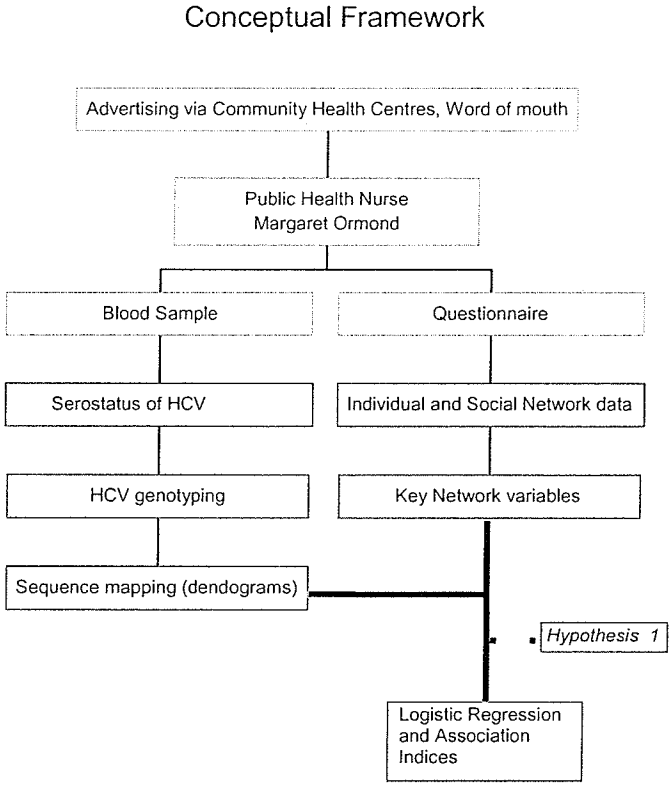
Data collection for all five aims, including for Aim 1, involved a survey questionnaire and blood sample. The conceptual framework (Figure 4) describes the study methods from recruitment up to the analysis of Aim 1. The initial recruitment for the study was through advertising the study by a public health nurse at various community health centers in the city and subsequently word of mouth within the injection drug community. The research study nurse was Margaret Ormond of Ormond Consulting Company. Ms. Ormond is known and trusted in the community and has a good knowledge of the injection drug use populations in Winnipeg. She has previous experience working with the targeted population in previous studies and in her capacity as a registered nurse. Ms. Ormond is well-known and respected within the IDU population, by community health centers and within the research community.

Eligibility criteria for the study included self-reported use of injection drugs in the last 6 months and a minimum age of 15 years. Data collection included a survey questionnaire (structured interview format) and a blood sample (Figure 4). Initial recruitment of participants involved advertising at community health centers and actively approaching potential participants to inform them of the study (Figure 4). Following initial advertising efforts, recruitment by word of mouth amongst the injection drug use community was employed. To ensure voluntary participation, potential participants self-initiated contact with study personnel. The location of the meeting was determined at the discretion of the participant and Ms. Ormond. Prior to administering the questionnaire, the participant was asked if they were interested in providing a blood sample for the

study. The participant had the opportunity to opt-out of specimen provision without jeopardizing their forty-dollar honorarium. Participants were made aware of the option of voluntary withdrawal during the questionnaire or blood sample donation, and that withdrawal from the study would not affect their receipt of the honorarium. Consent for blood testing (for HCV) was obtained separately from consent for molecular typing of HCV for use in the study.

The overall study, including aims and objectives of this thesis, was submitted for Ethics approval to the Health Research Ethics Board (HREB) of the University of Manitoba. Subsequently, I also submitted and obtained Ethics approval from the HREB of the University of Manitoba for the research that I conducted (Objectives 1-3) as a requirement of the Department of Community Health Sciences Master's degree program.

Figure 4: Conceptual framework of the study design



3.2 Data collection and analysis

3.2.1 Survey questionnaire

The survey questionnaire was designed to collect relevant social data and to generate individual and network variables for analysis (Figure 4). Results of the questionnaire were coded and entered into a computerized database in Microsoft Access.

Margaret Ormond administered the survey questionnaire with IDU who agreed to participate in the study. The purpose of the cross-sectional questionnaire (Appendix 1) was to collect the following information:

1. Individual demographics: including gender, ethnicity, residence
2. Individual drug behaviours: including drug preference, frequency and locations of injection
3. Needle sources: including availability of clean needles
4. Drug bingeing: including drug type and length of time of a binge
5. Smoking, inhaling or snorting drugs
6. Sexual behaviours: including same sex partner, and casual or client relationships
7. Health and social support: including social support, social diversity, drug dependency, depression, extraversion, infection information for study participant, overall group norms
8. Social network information.

The social network section included listing the study participant's network members (to a maximum of 20 people), their relationship to network members, and density of network (i.e. which network members knew each other). Network members were defined as people with whom participants had seen or spoken with on a regular basis in the previous 30 days. The questions on the social network contact list included

the gender, ethnicity, relationship of each contact, as well as whether the participant believed the contact was an IDU and/or a snorter/inhaler of drugs.

Detailed questions were asked about five of the individuals listed as network members who, to the knowledge of the participant, use injection drugs. The questionnaire was field tested on 5 IDU before implementation of the study. The questionnaire was formulated with the input of Dr. Samuel R. Friedman, a social epidemiologist who has worked with social network analysis and Injection drug users in New York [40, 73] (from the Research Proposal of John Wylie).

From this point on, the data collected from section 1-7 of the survey questionnaire described above will be referred to as *Individual data*. *Egocentric network data* will refer to the listing of all contacts and the *Risk network data* will refer to the section of the questionnaire asking in depth questions regarding up to 5 IDU in the participants contact list. If study participants listed more than 5 IDU in the *Egocentric network data* section, the first 5 IDU were selected, in order that they were named, for the *Risk network data* section.

3.2.2 Data management

The data was entered into the Winnipeg IDU SNS database in 4 sections: the first section contained the *Individual data*; the second section contained the infection status based on results including HCV results as diagnosed by Serology section of CPL, the third section contained *Egocentric network data* and the fourth section was for *Risk network data.*

Data cleaning was done in Microsoft Access, Microsoft Excel and STATA version 8.0 [74]. Frequency distributions for all data were generated in STATA and then

revised manually for errors. When a question's response included an "other" category, the responses were reviewed and the most frequently mentioned responses were kept as separate categories, while those responses only mentioned a few times were grouped together in an "other" category or considered outliers and removed from further analysis.

3.2.3 Contact data

Apart from the database containing the *Individual data*, the two additional databases, *Egocentric network data* and *Risk network data* required further preparation prior to statistical analysis. For purposes of my research, these databases were summarized into single lines of data (from up to 20 lines or up to 5 lines in original databases). Both condensed contact databases were linked by study participant codes to individual data.

3.2.4 Univariate Analysis

For analyses addressing the correlates of HCV seroprevalence the dependent variable was HCV status (coded as 1 for HCV positive and 0 for HCV negative). For analyses addressing the correlates with specific genotypes the dependent variable was the type of HCV infecting a study participant (coded as 1 for type 3a and 0 for type 1a). The Chi-squared test (x^2) and Fisher's exact test were used to test univariate significance of the categorical individual data. Linearity of continuous variables was also tested.

3.2.5 Correlation Matrix

The individual data, and collapsed contact data univariately associated with the outcomes were run simultaneously in a correlation matrix in STATA [74]. The matrix was created by computing the correlation coefficients between all variables included in STATA. For example, correlating variables a, b and c, will determine the correlation

between a and b, a and c, and b and c. Correlations of 0.70 or greater were evaluated and either combined in a meaningful way, or one of the two correlated variables were dropped based on assessing which was potentially more meaningful [75]. The correlation matrix value is also comparable to the Phi value or Kramer's V value in measuring the strength of correlation between two categorical variables.

3.2.6 Multivariate analysis

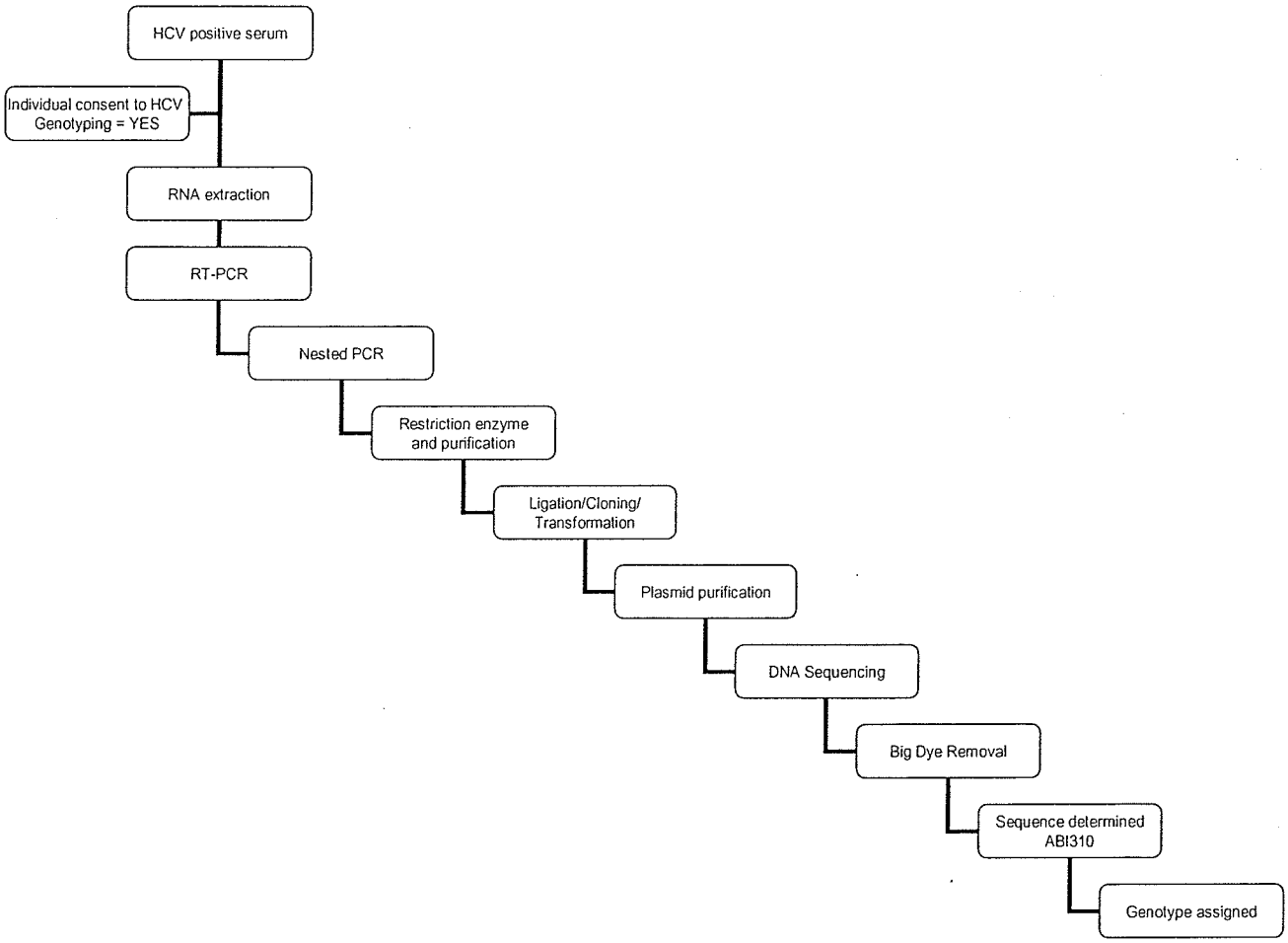
Based on results of univariate analysis, variables were retained for multivariate level analysis. Variables significant at the 0.10 level were entered into multivariate analysis [76]. The decision to include certain variables was based primarily on p values at the univariate-level. Comparison of models was based on the -2 log likelihood ratio. The test involved removing a variable and comparing the reduced model to the full model by comparing the overall Chi-square value of the models to detect a significant difference. In this test, coefficients were also monitored for changes. Hosmer-Lemeshow goodness-of-fit tests were utilized to assess the significance of the final models. All variables significant in the final model were tested for potential significant interactions. STATA version 8.0 [74] was used for statistical analysis. The events per parameter, also known as the rule of ten, was used to ensure model fit, such that the final model would have no more parameters than the frequency of the least frequent outcome variable per 10 observations [76].

3.3 Molecular methods

3.3.1 Overview of Molecular methods

Figure 5 conceptually displays the steps from an HCV positive specimen to the generation of DNA sequence data.

Figure 5: Overview of Molecular methods from HCV serum to Genotyping.



3.3.2 Sample collection and storage

Serum samples were submitted to the serology section of CPL for clinical diagnosis of HCV infection status. Serum specimens were screened for the presence of antibodies against HCV antigens with AxSYM HCV (Abbott, Mississauga, ON) followed by confirmation with Chiron HCV 3.0 RIBA (Ortho-Clinical Diagnostics, Markham, ON).

Samples were initially stored at 4°C, after centrifugation and aliquoting of sera by the Serology section of CPL. Any surplus serum remaining after HCV testing was retrieved from the Serology section and frozen at –70°C in 500 μL aliquots until further molecular work was performed.

3.3.3 Viral RNA Isolation, cDNA Synthesis and PCR amplification

RNA was extracted from 140 μL of HCV-positive serum with QIAamp Viral RNA extraction kit (Qiagen, Mississauga, ON). For reverse transcription the SuperscriptIII First-Strand Synthesis System for RT-PCR was used (Invitrogen, Burlington, ON). Eight μL RNA extract was incubated with 1 μL Random hexamers and 1 μL dNTP for 5 minutes at 65°C followed by cooling on ice for 5 minutes. The negative control followed the same procedure, containing 1 μL Random hexamers, 1 μL dNTP, and 8 μL DepC H_20 (from the Superscript III kit) instead of RNA extract (DepC H_20 is deionized water suitable for use with RNA, as it inactivates RNase). Subsequently, 10 μL of the reverse transcription mixture (2 μL of 10xRT buffer, 4 μL $MgCl_2$, 2 μL DTT, 1 μL RNase out and 1 μL Superscript III enzyme) was added to each sample. The samples were then subjected to Steps 3-6 in Table 2.

Following RT-PCR, cDNA was subjected to a Two-step Nested PCR. The first PCR was performed in a 50μL volume containing 39.91μL H_2O, 5μL buffer with 15 mM $MgCl_2$ (Finnzymes, Ipswich, MA), 0.4μL dNTP (25mM), 0.09 pmoles HCV E1 primer, 0.08 pmoles HCV E2 primer, 1 μL (2 Units) Dynazyme enzyme (Finnzymes, Ipswich, MA) and 3 μL of study participant sample cDNA. The PCR reaction conditions are described in Table 4. Three μL of the first PCR reaction mix were transferred to a second PCR reaction mixture of 80.66μL H_2O, 10μL buffer with 15mM $MgCl_2$, 0.8μL dNTP, 0.21 pmoles HCV E3 primer, 0.18 pmoles HCV E4 primer, 2μL Dynazyme enzyme (Finnzymes, Ipswich, MA). The second PCR conditions were the same as those for the first PCR reaction.

Table 3 displays all PCR primers used in this study. These primers are discussed in Garson *et al.*(1990) and Corbet *et al.*(2003) [77, 78]. HCV Primers for nested PCR were E1-4 reverse and forward (Core to envelope 1 region) [77]. All PCR primers were manufactured by University Core DNA Services at the University of Calgary (Calgary, AB).

In certain instances, if PCR products were initially undetectable, double extractions were performed and if still undetectable, conserved primers were used (HCV NCR 1 – 4 in Table 3). If following double extraction and PCR with NCR primers, product was undetected, the sample was considered unusable, due to low viral load, and was not used further. The two sets of protocols in Table 4 are for two separate thermal cyclers. The choice of thermal cycler was made based on availability, as these instruments are shared with other users.

Fifteen μL of the nested PCR product was analyzed by electrophoresis on a 1% agarose gel (Bio-Rad Laboratories, Hercules, CA) in a 5x Tris-boric acid-ethylenediamine tetraacetic acid buffer (5xTBE). PCR products were visualized with ethidium bromide. The expected size of the final PCR product was 424 base pairs (bp).

All RT-PCR and Nested PCR reactions were prepared in dedicated PCR preparation areas at CPL. All precautions were taken to avoid any possible contamination, using separate locations for extraction of specimen, addition of DNA for first and second PCR, amplification of HCV RNA, and analyses of PCR products. Negative and Positive controls were used in all PCR reactions to monitor for contamination and for detection of PCR failures.

3.3.4 DNA Ligation, Transformation and Plasmid purification

PCR products were purified using a Qiaquick kit (Qiagen, Mississauga, ON) following manufacturer's instructions. A Minelute kit (Qiagen, Mississauga, ON) was used to purify and concentrate PCR products if they appeared as very faint bands on the agarose gels. Forty-five μL of PCR product was typically prepared for cloning by digestion with 25 units of *Eco*RI (Invitrogen, Burlington, ON) and 25 units of *Bam*HI (Invitrogen, Burlington, ON). The pGEM vector (Promega, Nepean, ON) was also cleaved using 50 μL H_20, 1x React 3, 20 units *Eco*R1, 20 units *Bam*H1, 20 μL pGEM vector (from pGEM®-T vector system kit, Promega, Nepean, ON) and incubated in a 37°C water bath for one hour. Subsequently, 5 μL of digests and 5 μL tracking dye were loaded onto 1% agarose gel (Bio-Rad Laboratories, Hercules, CA) to verify digestion. The pGEM vectors were cleaved in batch quantities and stored at -70°C until required.

The molecular weight standards used for DNA fragment sizing were the Low DNA Mass Ladder (Invitrogen, Burlington, ON), which allowed estimates of both fragment size and quantity.

A 3:1 insert to vector ratio (~3-30 fmoles vector and 9-90 fmoles PCR insert) was used for ligation. Ligation was completed with 1 unit T4 DNA ligase (Invitrogen, Burlington, ON) and incubation at room temperature for 1 hour. Following ligation, transformation of competent cells was carried out. Initially, JM109 (Promega, Nepean, ON) competent cells were used. Due to inefficiency in transformations, protocols were changed to use Subcloning Efficiency DH5α Competent Cells (Invitrogen, Burlington, ON). Protocols for using both types of cells remained the same. Twenty-five μL of competent cells were thawed on ice and aliquoted into separate tubes. One μL of a ligation reaction was added to the cells and gently mixed by flicking. Tubes were incubated on ice for 5 minutes, followed by incubation in a 42 ° C water bath for 30 seconds, then returned to ice for 5 minutes. One hundred μL of SOC medium (Invitrogen, Burlington, ON) was added to each transformation tube. An aliquot of 100 μL was plated on LB agar (Difco Laboratories, Sparks, MD) plates and incubated at 37°C overnight. Every 1000 mL of LB agar contained 1 ml of 100 mg/mL ampicillin (AMP), 2 mL of 100 mM isopropyl-ß-D-thiogalactoside (IPTG), and 0.5 mL of 40 mg/mL 5-bromo-4-chloro-3-indolyl-ß-D-galactose (XGAL).

Insert-containing plasmids were identified by standard blue/white (Xgal/IPTG) selection. Individual colonies appearing white, indicating the possible presence of insert, were selected with a sterile loop and re-isolated on LB agar at 37°C. Cells were

subsequently incubated (shaking incubator) in 5 mL of TS broth with 5 μL of AMP overnight at 37 ° C in preparation for purification of plasmids.

The Wizard Plus SV miniprep plasmid purification kit (Fisher, Norcross, GA) was used for purification of potential insert-containing plasmids. Plasmids were digested in a 20 μL reaction volume containing 5 μL of purified plasmid, 1xReact3 Restriction Enzyme buffer, 5 Units *Eco*R1 and 5 Units *Bam*H1. The reactions were incubated at 37°C for one hour and the presence of inserts was verified on 1% agarose gels.

Three insert containing clones per available HCV positive serum were sequenced to determine the genotype of each HCV positive study participant.

3.3.5 DNA Sequencing

To determine the genotype of HCV, purified plasmids were sequenced using ABI Prism Big Dye™ termination kit (PE Applied Biosystems, Mississauga, ON) and analyzed using an ABI 310 genetic analyzer (PE Applied Biosystems, Mississauga, ON). Sequencing reactions used either forward primer M13F: GTAAAACGACGGCCAGT or reverse primer M13R: AACAGCTATGACCATG (University Core DNA Services, University of Calgary, Calgary, AB). Sequencing reaction followed manufacturer's instructions and are outlined in Table 5.

Big Dye Removal Kits (Qiagen, Missisauga, ON) were used for clean-up of sequencing reactions as per manufacturer's instructions. Following Big Dye Removal protocols reaction mixes were immediately loaded onto the ABI 310 Genetic Analyzer (PE Applied Biosystems, Missisauga, ON). Initially, DNA to be analyzed on the ABI 310 was resuspended in 17 μL of TSR buffer (PE Applied Biosystems, Missisauga, ON), but due to changes in the manufacturer's protocols, resuspension buffer was later

changed to 17 μL of HI-DI formamide (PE Applied Biosystems, Missisauga, ON). This facilitated DNA resuspension as it negated the need for incubations at 95°C and 4°C (PE Applied Biosystems, Mississauga, ON). All laboratory procedures were completed at CPL (Winnipeg, Canada).

3.3.6 DNA sequence manipulation and phylogenetic analysis

Three clones were selected to determine the genotype of each study specimen. Controversy exists as to the representativeness of a few clones in the variety of sequences present in an HCV infected individual. A study of the minimum number of clones to sequence for the maximum information on quasispecies suggested that selecting three to five clones was not as informative as selecting 40 clones to understand pathogenesis [80]. However, the study also argues that the thorough investigation of quasispecies is to better understand pathogenesis, particularly where carcinomas are concerned. The use of clones in this study is to address the most variable region of the genome and the focus is to determine genotype and subsequently assess transmission patterns. This study does not examine the degree of virus evolution or viral divergence within individuals, or the rate of pathogenesis, where inclusion of more clones would be necessary. The decision to use three clones was also to maintain a feasible work load within the study time lines, while producing sufficient information regarding genotype and viral divergence present in our study population.

Alignment of forward and reverse sequences was completed with AlignPlus (Scientific & Educational Software, Cary, NC). Primer sequences were removed and the final consensus sequences were entered into Bionumerics software version 3.5 (Applied Maths Inc., Austin, TX) for comparison to reference genotypes.

Reference genotypes were obtained from Los Alamos database. Accession numbers for reference sequences are as follows: 1a [AF290978, D10749, M67463], 1b [D10934, AJ000009]; 1c [AY051292, D14853]; 2a [AF169004, AF169002]; 2b [AF238486, D10988, AB030907]; 2c [D50409]; 2k [AB031663]; 3a [D28917, D17763, X76918]; 3b[D49374]; 3k [D63821]; 4a [Y11604]; 5a [Y13184, AF064490]; 6a [Y12083]; 6b [D84262]; 6d [D84263]; 6g [D63822]; 6h [D84265]; 6k [D8426]. Details of genotype and subtype designation, nomenclature and reference genotype set are decided upon by a panel of experts and are published in peer-reviewed literature [81]. New genotypes are assigned provisionally if they are phylogenetically different from other genotypes and confirmed by two HCV variants from infections which are not linked epidemiologically. Subtype designations are based on three or more examples of infections.

Phylogenetic trees were drawn based on maximum evolutionary distances using Bionumerics (Applied Maths Inc., Austin, TX) with reference sequences and test sequences to determine genotype. A maximum parsimony dendrogram was also drawn using Bionumerics (Applied Maths Inc, Austin, TX).

3.3.7 Association indices (AI)

Procedures used for the scoring of AI values are derived from methods and theory described first in Wang *et al.* (2001) and subsequently in Cochrane *et al.* (2002), and Van Asten *et al.*(2004) [28, 52, 64]. The programs used for the Association indices analysis were: ClustalW, PHYLIP, Simmonics Sequence Editor, MEGA [62, 82-84]. The method requires installation of PHYLIP programs DNADIST, NEIGHBOUR and SEQBOOT, and requires that output files be stored in the same directory as the PHYLIP programs

[83]. PHYLIP software is necessary for the drawing of phylogenetic trees and for calculation of associations.

After genotype determination, one sequence (of the three cloned) per study participant was selected for further AI analysis. To correlate behaviour and demographic characteristics of study participants to their sequence data, only one sequence per individual study participant could be entered into analysis. Additionally, the AI analysis assesses relationship between individuals (not within individuals), and selection of one clone per participant was sufficient for analysis of relatedness of HCV between individuals. To select which of the three sequences should be considered, a phylogenetic tree based on maximum evolutionary distances of all test sequences was examined. Each study participant's sequences were visually compared to those of neighbouring study participants. In most instances, a single study participant's sequences clustered closely together and distinct from other study participants, the sequence that was most homologous to the two other sequences in the cluster for the study participant was selected (i.e. best representing the cluster). However, in a few instances where two or more study participants' sequences were closely grouped, the sequence that connected closest with another study participants sequences was selected for each individual, to maximize the likelihood of finding a relationship between closely related sequences between individuals.

The sequences selected were aligned in ClustalW [82]. The output file was stored in Phylip format [83] as required for use in Simmonics Sequence Editor 2000 version 1.3. An epidemiologically unlinked (outgroup) reference sequence was included with test sequences prior to input into Clustal W, for comparison during AI analysis. Consensus

sequence genotype 2a, selected because of its distinctness from 1a and 3a sequences in a maximum likelihood phylogenetic tree, was used as the outgroup sequence for all AI calculations.

Prior to generating the AI values, the aligned sequences were labeled (numerically tagged) based on selected variables, in the Simmonic 2000 Sequence Editor package [62]. Selected variables labeled included location of injection (hotel, shooting gallery, on the street), geographic information (where participant lives and hangs out, whether they recently moved to Winnipeg), demographic information (gender, ethnicity, age), drug choice (drug type), contact information (number of contacts, type of contact). For each analysis, the mean evolutionary distances between sequences within each variables' category and between all sequences were calculated by Simmonic 2000 Sequence Editor Package [62].

Although the significance cannot be calculated, the confidence intervals give an indication of when there is a significance difference. The output values generate percentile ranges for the test sequences and for the control sequences, based on bootstrap resampling. If the Upper percentile for the test sequences is greater than the lower of the control sequence, then no significant difference exists between segregation in test sequences when compared to random control sequences.

From ClustalW, sequence files were also saved for input into MEGA software. The files were converted to MEGA format, and phylogenetic trees were drawn using Neighbour-joining method.

Table 2: Reverse transcription steps for thermocycler

	temperature (PTC-100)	
Step 1	65°C	5 min
Step 2	Ice	5 min
Step 3	25°C	50 min
Step 4	50°C	
Step 5	85°C	
Step 6	4°C hold	

Table 3: Nested PCR primers for HVR 1 region and for conserved region

Primer	Position (nt*)	Nucleotide sequence, 5' to 3'
HCV E1 reverse	493-518	GCAACAGGGAACCTTCCTGGTTGCTC
HCV E2 forward	987-964	CGTAGGGGACCAGTTCATCATCAT
HCV E3 reverse	502-527	CGGGATCCTTCCTGGTTGCTCTTTCTCTAT
HCV E4 forward	975-952	GGAATTCATCATCATATCCCATGCCAT
HCV NCR1 reverse	1-20	GTATCTCGAGGCGACACTCCACCATAGAT
HCV NCR2 forward	323-303	ATACTCAGGTGCACGGTCTACGAGACCT
HCV NCR3 reverse	10-31	CGGGATCCACCATAGATCTCTCTCCCCTGT
HCV NCR4 forward	296-271	GGAATTCACTCTCGAGCACCCTATCAGGCAGT

*As per Corbet *et al.*, E1-E4 primers numbered according to the first nucleotide in the ORF of the HCV reference strain H77 (See GenBank accession no. AF009606)[77]. For NCR1-4 as per Garson *et al*, primers numbered according to numbering system of Okamoto *et al.* [78, 79].

Table 4: Thermocycler programs for Nested PCR

	Nested PCR (PTC-100)	Nested PCR (9600)	
Step 1	95°C for 9 min	95°C for 5 min	
Step 2	95°C for 60 sec	95°C for 40 sec	30 cycles
Step 3	50°C for 60 sec	50°C for 40 sec	
Step 4	72°C for 90 sec	72°C for 90 sec	
Step 5	72°C for 6 min and 30 sec.	72°C 5 min	
Step 6	4°C hold	4°C hold	

Tal[illegible] **mocycler program for Sequencing Reaction**

Sequencing Reaction (9600 Thermocyler)	
96°C for 10 sec	25 cycles
50°C for 5 sec	
60°C for 4 min	
4°C hold	

3.4 Review of methods and analytic strategy

The methods described above, were used in various combinations to analyze data for Objectives 1-3. A 3-stage hierarchical approach was used where step 1 analyzed the correlates between social data and HCV status (positive or negative). This step provides general data on the possible risk factors associated with infection with HCV in general, regardless of genotype. Step 2 utilized data from the subset of individuals who were HCV positive. This step analyzed the correlations between social variables and the two most common genotypes of HCV found in this study (genotypes 1a and 3a). This step determined whether these two genotypes of HCV may be circulating in different groups of IDU (i.e. different transmission networks). Both steps 1 and 2 used logistic regression analysis to determine correlates. Finally, step 3 utilized the full extent of the DNA sequence data generated from the HCV positive specimens. This step, through the use of association indices, allowed us to analyze data at the most detailed level (i.e. division of genotypes into subgroups). This final approach was undertaken as it could reveal transmission networks that would not otherwise be visible by carrying out only broader level analyses (steps 1 and 2).

Chapter 4:Results

4.1 Description of study population

The in-depth description of this study population has been discussed in a published report by Manitoba Health (Appendix 3). As such, only select descriptive statistics are discussed in this section. A total of 435 IDU were interviewed for the study.

The mean age of participation was 34.9 years (SD 9.9). The participants included 241 (55.7%) males and 186 (43.0%) females with an additional 6 (1.4%) transgender females. Transgender females were all biological males presenting as females.

Four hundred and nineteen participants were born in Canada, of which 174 (40.2%) were born in Winnipeg, 132 (30.5%) were born in other parts of Manitoba and 113 (26.1%) were born in Canada but outside Manitoba. The remaining 14 (3.2%) participants were born outside Canada. In total 306 (70.7%) of the participants were Manitoban born.

Two hundred and ninety-eight (68.8%) of the participants reported dropping out of high school. In comparison, 69 (15.9%) reported higher educational levels (including University and College). Of those reporting high school dropout, 148 (50.0%) specified dropping out in or above grade 10 while 150 (50.3%) specified dropping out in or below grade 9. Welfare, EI, Pension or other government support was reported by 64.4% as main source of income.

Self-reported ethnicity showed 204 (47. 1%) as First Nations (Treaty/Non treaty), 149 (34.4%) as Caucasian, and 68 (15.7%) identifying themselves as Métis. An

additional 12 (2.8%) participants reported themselves as belonging to other ethnic groups including Latin American, Middle Eastern, Black-Caribbean, and Mixed ethnicities.

The mean age for first injection was 20.8 years (SD 7.2). The most commonly reported preferred injection drugs were Cocaine (168, 38.8%) followed by Talwin and Ritalin (87, 20.1%), Heroin (57, 13.2%) and Morphine (39, 9.0%). In comparison, the most frequently injected drugs were Cocaine (157, 36.4%),Talwin and Ritalin (103, 23.9%) and Morphine (7, 16.2%). In this study, the preferred drug entails the drug that would be injected if cost and availability were not obstacles, whereas most frequently injected drug refers to the drug that the study participant uses most often.

For how often the participant injected in the past month, 107 (24.8%) reported "not at all", and 126 (29.2%) reported "once in a while, not every week", while 71 (16.5%) reported injection "every day", 67 (15.6%) reported "regularly, once or twice a week", and 58 (13.5%) reported "regularly, 3 or more times a week". Four hundred and nine (94.5%) participants reported injecting in a private residence (including residences of family or friends. One hundred and ten (25.0%) participants reported having injected in a public place such as parks, bars, or washrooms.

From a network perspective, the mean number of contacts named was 8.7, with a range from 0 to 20. Although twenty was the maximum number, 18(4.2%) study participants were prepared to list more than 20 contacts. The mean age of network members was 34.0 years. Three hundred and eighty-six (90.0%) study participants had network members who were IDUs. The average number of IDU network members was 6.4 IDU.

4.2 Correlated variables

Table 6 shows variables that were correlated at the 0.70 level in or higher in the correlation matrix and the alterations that were made to these variables. The correlation matrix is discussed in section 3.2.5 of the Methods section.

In most instances, highly correlated variables were similar in meaning and the more pertinent was kept in analysis. For example, participant age and length of injection were correlated, so only participant age was retained. In the case of Talwin and Ritalin use, an individual's self-reported use of Talwin and Ritalin was correlated with whether their network members reportedly used Talwin and Ritalin. A new variable defined whether study participants either had no connection to Talwin and Ritalin use or were linked to Talwin and Ritalin use (either through their own use or by associating with a network member who used Talwin and Ritalin). Individual and network use of other drug types were not correlated at the 0.70 level and were not combined in this manner.

Table 6: Correlated variables from correlation matrix

Variable	Decision	Correlation
Birthplace city; Birthplace province	Dropped province, Kept birthplace city (recatagorized into Winnipeg, In Manitoba (outside of Winnipeg), In Canada (outside Manitoba), Foreign born	0.74
Individual use of Talwin and Ritalin in the past 6 months; IDU Network member use of Talwin and Ritalin	Combined to reflect individual and/or network member use of Talwin and Ritalin (See text above for detailed explanation)	0.76
Individual age; Length of injection	Dropped length of injection	0.74
Got needles from a needle exchange; Got needles from Street Connections	Kept Got needles from Street Connections (due to specificity)	0.71

4.3 Results for Objective 1: Correlate the seroprevalence of HCV with individual and social network variables.

4.3.1 Univariate and multivariate results for Objective 1

The sample size for the study was 435 participants. Of the total sample, results were not available for 51 specimens. Of these 51, 2 specimens were indeterminant for antibody to HCV, 3 specimens were of insufficient quantity to permit testing and 46 participants refused or were unable to provide blood specimen. Therefore, data used for the analysis of Objective 1 consisted of the 384 participants who were determined to be either seronegative or seropositive for HCV (209 seropositive [54.2%], 176 seronegative [45.8%]). Subsequently three of these participants (2 HCV positive, 1 HCV negative) did not complete study questionnaire, and were removed.

The variables used in the analysis for this thesis include those behaviours directly resulting in HCV transmission, for example, sharing needles, or other injection equipment such as cottons, cookers or rinse water. The latter behaviours were queried regarding the previous 6 months; however, whether IDU had "ever" shared needles was also included. Other variables, such as ethnicity, gender, age and drug choice, although not directly resulting in HCV acquisition, have been shown to influence risk behaviours and were also included in the analysis [85-88]. Injection sites, such as hotels, were included, as previous analysis has shown social venues to be important in transmission of infectious pathogens [89, 90].

The social network variables selected for analysis included the drug-related behaviours exhibited by the IDU within a study participant's risk network, such as: type of drugs injected, injection sites frequented and frequency of injection. Also included

were the type of relationships that existed between study participants and their network IDUs, and the number of IDUs in a study participant's egocentric network.

Univariate results correlating sociodemographic and behavioural data with the dependent variable (HCV serostatus) are provided in Table 7. At the univariate-level, Chi-square test was used for all categorical variables.

Of the 385 specimens obtained in the study, 54.4% (209) were HCV seropositive. For individual characteristics, the following correlates of HCV infection were identified (for simplicity only univariate results with $p<0.05$ are listed; although as noted above, a less stringent criteria was used for entry into multivariate analysis): older age, initiation into drug use at a younger age, Aboriginal or Métis descent, frequent injection, injection at a shooting gallery, having used someone else's used needle, having injected someone else, and having obtained needles from needle exchanges or pharmacies. Individuals who have sold drugs were less likely to be HCV positive. The social network variables correlated with a greater likelihood of HCV infection included: having other family members who were IDU, associating with IDU who inject at hotels, and being linked to Talwin and Ritalin use.

Variables significant at the $p<0.100$ level in univariate analysis were entered into multivariate analysis. The final model was created by removing variables that did not significantly change overall model goodness-of-fit, and therefore were not significantly associated with HCV outcome when compared alongside other variables. The final model, containing only variables significant at $p<0.05$, is displayed in (Table 8). The final model was not adjusted for gender or ethnicity as neither significantly altered the overall goodness of fit for the model. The Hosmer-Lemeshow goodness of fit test for this

model was 0.71. HCV positivity was correlated with older age, younger age of initiation to injection drug use, injecting at a shooting gallery, having injected someone else as a service, injection with a used needle, obtaining needles from a mobile exchange site and being associated with Talwin and Ritalin use.

4.3.2 Significant Interactions for Objective 1

The effect of the interaction between sharing needles and Talwin and Ritalin use was statistically significant (Table 9). For IDU not associated with the use of Talwin and Ritalin, using other IDUs' previously used syringes is correlated with HCV positivity, represented by a confidence interval that does not cross over 1. However, this correlation was absent among IDU who are associated with Talwin and Ritalin use. For this group of IDU, there was no significant difference in HCV positivity between those IDU who have reported that they have injected with used syringes and those IDU who do not report this behaviour. This relationship is also evident in Table 10, in a breakdown of participants.

Table 7: Univariate-level analysis for HCV outcome

		HCV negative N (%)	HCV Positive N (%)	Odds Ratio	95% Confidence Interval	p value
Total subjects		175 (45.8)	207 (54.2)			
Demographics						
Age	*Mean (SD)*	31.2	38.4	2.24*	1.77 to 2.85	<0.001
Gender						
	Male	101(46.5)	116 (53.5)	1.00		
	Female	71 (44.4)	89 (55.6)	1.09	0.72 to 1.65	0.677
Ethnicity						
	Caucasian	71 (55.0)	58 (45.0)	1.00		
	First Nation (treaty/non-treaty)	77 (42.3)	105 (57.7)	1.67	1.05 to 2.64	0.027
	Métis	22 (36.1)	39 (63.9)	2.17	1.15 to 4.11	0.015
	Other	5 (50.0)	5 (50.0)	1.22	0.34 to 4.46	0.758
Birthplace						
	Winnipeg	61 (39.6)	93 (60.4)	1.00		
	rural Manitoba	53 (43.8)	68 (56.2)	0.84	0.52 to 1.37	0.485
	Canada (outside Manitoba)	55 (58.5)	39 (41.5)	0.47	0.27 to 0.79	0.004
	Outside Canada	6 (46.2)	7 (53.8)	0.77	0.24 to 2.40	0.645
Primary source of income						
	Regular work	46 (54.8)	38 (45.2)	1.00		
	Financial support (Government/friends or family)	100 (40.0)	150 (60.0)	1.82	1.10 to 3.00	0.019
	Illegal activities	27 (58.7)	19 (41.3)	0.85	0.41 to 1.77	0.67
Other sources of income over the last year:						
Regular work	*No*	99 (41.9)	137 (58.0)	1.00		
	Yes	76 (52.1)	70 (47.9)	0.67	0.44 to 1.01	0.054
Welfare	*No*	127 (44.7)	157 (55.3)	1.00		
	Yes	48 (49.0)	50 (51.0)	0.84	0.53 to 1.33	0.466
Money from family or friends	*No*	75 (41.4)	106 (58.6)	1.00		
	Yes	100 (49.8)	101 (50.2)	0.71	0.48 to 1.07	0.104
Sex trade	*No*	150 (49.2)	155 (50.8)	1.00		
	Yes	25 (32.5)	52 (67.5)	2.01	1.82 to 3.43	0.009
Dealing drugs	*No*	87 (44.2)	110 (55.8)	1.00		
	Yes	88 (47.6)	97 (52.4)	0.87	0.58 to 1.31	0.505
Panhandling	*No*	151 (47.2)	169 (52.8)	1.00		
	Yes	24 (38.7)	38 (61.3)	1.41	0.81 to 2.47	0.221
Boost or Stealing	*No*	123 (51.9)	114 (48.1)			
	Yes	52 (35.9)	93 (64.1)	1.93	1.26 to 2.95	0.002
Education						
	Graduated high school	11 (28.2)	28 (71.8)	1.00		
	In grade school now	8 (50.0)	8 (50.0)	0.39	0.11to 1.36	0.126
	Dropped out in or before grade 9	67 (44.0)	79 (56.0)	0.50	0.23 to 1.09	0.077
	Dropped out between grade 9 and grade 12	58 (46.4)	67 (53.6)	0.45	0.21 to 1.00	0.045
	Higher Learning	36 (59.0)	25 (41.0)	0.27	0.10 to 0.68	0.003
Number of places lived in the last year	*Scale*			0.86	0.77 to 0.97	0.014
Moved to Winnipeg in the past year	*No*	118 (42.6)	159 (57.4)			
	Yes	57 (54.3)	48 (45.7)	0.62	0.40 to 0.98	0.041
Drug use related behaviours						
Age of first injection	*Mean (SD)*	21.9	20.2	0.86†	0.75 to 0.99	0.028
Non-injection drugs:						
Alcohol	*No*	17 (42.5)	23 (57.5)	1.00		
	Yes	158 (46.2)	184 (53.8)	0.86	0.44 to 1.67	0.657
Acid	*No*	149 (44.0)	190 (56.0)	1.00		

	Yes	26 (60.5)	17 (39.5)	0.51	0.27 to 0.98	0.040
Painkillers	*No*	93 (46.5)	107 (53.5)	1.00		
	Yes	82 (45.1)	100 (54.9)	1.06	0.71 to 1.59	0.777
Amphetamines	*No*	131 (43.2)	172 (56.8)	1.00		
	Yes	44 (55.7)	35 (44.3)	0.61	0.37 to 1.00	0.048
Barbiturates	*No*	78 (46.2)	91 (53.8)	1.00		
	Yes	97 (45.5)	116 (54.4)	1.03	0.68 to 1.54	0.905
Cocaine	*No*	52 (43.7)	67 (56.3)			
	Yes	123 (46.8)	140 (53.2)	0.88	0.57to 1.37	0.578
Crack	*No*	41 (49.4)	42 (50.6)			
	Yes	134 (44.8)	165 (55.2)	1.20	0.74 to 1.95	0.459
Demerol/morphine/opium	*No*	102 (47.2)	114 (52.8)			
	Yes	73 (44.0)	93 (56.0)	1.14	0.76 to 1.71	0.528
Downers/Tranquilizers	*No*	63 (52.1)	58 (47.9)	1.00		
	Yes	112 (42.9)	149 (57.1)	1.45	0.93 to 2.23	0.095
Ecstasy	*No*	155 (43.7)	200 (56.3)	1.00		
	Yes	20 (74.1)	7 (25.9)	0.27	0.11 to 0.67	0.002
Gasoline/Solvents	*No*	164 (48.8)	172 (51.2)	1.00		
	Yes	11 (23.9)	35 (76.1)	3.03	1.48 to 6.24	0.002
Marijuana	*No*	30 (36.6)	52 (63.4)			
	Yes	145 (48.3)	155 (51.2)	0.75	0.31 to 1.85	0.535
Tylenol #3	*No*	61 (46.9)	69 (53.1)			
	Yes	114 (45.2)	138 (54.8)	1.07	0.70 to 1.64	0.755
Heroin	*No*	153 (44.6)	190 (55.4)			
	Yes	22 (56.4)	17 (43.6)	0.62	0.32 to 1.22	0.162
Mushrooms	*No*	117 (40.6)	171 (59.4)			
	Yes	58 (61.7)	36 (38.3)	0.42	0.26 to 0.69	<0.001
Methadone (prescribed)	*No*	156 (47.7)	171 (52.3)			
	Yes	19 (34.5)	36 (65.5)	1.73	0.95 to 3.15	0.070
Methadone (unprescribed)	*No*	145 (48.2)	156 (51.8)			
	Yes	30 (37.0)	51 (63.0)	1.58	0.95 to 2.62	0.075
Crystal meth	*No*	134 (42.0)	185 (58.0)			
	Yes	41 (65.1)	22 (34.9)	0.39	0.22 to 0.69	<0.001
Other	*No*	151 (44.8)	186 (55.2)			
	Yes	24 (53.3)	21 (46.7)	0.71	0.38 to 1.33	0.283
If drug injected is same as preferred drug	*No*	39 (55.7)	70 (64.2)			
	Yes	136 (49.8)	137 (50.2)	0.56	0.36 to 0.89	0.013
Drugs injected in last 6 months:						
Cocaine	*No*	66 (47.5)	73 (52.5)			
	Yes	109 (44.9)	134 (55.1)	1.11	0.73 to 1.69	0.621
Talwin and Ritalin	*No*	140 (54.0)	119 (46.0)			
	Yes	35 (28.5)	88 (71.5)	2.96	1.84 to 4.76	0.000
Morphine	*No*	116 (47.3)	129 (52.7)			
	Yes	59 (43.1)	78 (56.9)	1.19	0.78 to 1.81	0.421
Heroin	*No*	153 (44.3)	192 (55.7)			
	Yes	22 (59.5)	14 (40.5)	0.54	0.27 to 1.09	0.083
Crack/Rock cocaine	*No*	138 (47.9)	150 (52.1)			
	Yes	37 (39.4)	57 (60.6)	1.42	0.88 to 2.28	0.149
Methamphetamine	*No*	145 (42.5)	196 (57.5)			
	Yes	30 (73.2)	11 (26.8)	0.27	0.13 to 0.57	<0.001
How often shot up in the past month	*Not at all,*	52 (54.7)	43 (45.3)	1.00		
	Once in a while, not every week	58 (52.7)	52 (47.2)	1.08	0.62 to 1.88	0.774
	Regularly, once or twice a week	28 (50.9)	27 (49.1)	1.16	0.60 to 2.27	0.652
	Regularly, three or more times a week	17 (30.4)	39 (69.6)	2.77	1.35 to 5.71	0.004
	Everyday	18 (28.6)	45 (71.4)	3.02	1.49 to 6.12	0.001
Injection sites:						
Empty house	*No*	155 (47.4)	172 (52.6)	1.00		
	Yes	20 (36.4)	35 (63.6)	0.93	0.53 to 1.66	0.814
Hotel	*No*	113 (49.6)	115 (50.4)	1.00		

	Yes	62 (40.3)	92 (59.7)	1.46	0.96 to 2.21	0.074
Shooting gallery	*No*	159 (49.1)	165 (50.9)	1.00		
	Yes	16 (27.6)	42 (72.4)	2.53	1.36 to 4.72	0.003
Rooming/Boarding house	*No*	127 (48.5)	135 (51.5)	1.00		
	Yes	48 (40.0)	72 (60.0)	1.41	0.91 to 2.19	0.123
On the street	*No*	120 (44.6)	149 (55.4)	1.00		
	Yes	55 (48.7)	58 (51.3)	0.85	0.55 to 1.32	0.468
Vehicle	*No*	133 (46.5)	153 (53.5)	1.00		
	Yes	42 (43.8)	54 (56.3)	1.12	0.70 to 1.78	0.640
Public Washroom	*No*	138 (45.2)	167 (54.8)	1.00		
	Yes	37 (48.1)	44 (51.9)	0.89	0.54 to 1.48	0.659
In a private residence (at own house, friend's house or family's house)	*No*	13 (68.4)	6 (31.6)	1.00		
	Yes	162 (44.6)	201 (55.4)	2.69	0.99 to 7.28	0.043
Used someone else's cooker, rinse water or cotton (past 6 months)	*Never*	113 (47.7)	124 (52.3)	1.00		
	Ever	53 (41.2)	76 (58.9)	1.31	0.85 to 2.02	0.227
Ever used someone else's needle	*No*	92 (144.0)	52 (36.1)			
	Yes	59 (29.8)	139 (70.2)	4.08	2.51 to 6.63	<0.001
Sold drugs	*No*	79 (40.3)	117 (59.7)			
	Yes	95 (52.2)	87 (47.8)	0.62	0.41 to 0.93	0.020
How many times injected someone else for drugs money or other goods	*Never*	151 (53.2)	133 (46.8)			
	Ever	24 (24.7)	73 (75.3)	3.45	2.02 to 5.90	<0.001
How many times injected someone else with drugs as a favour	*Never*	122 (55.5)	98 (44.5)			
	Ever	53 (33.1)	107 (66.9)	2.51	1.63 to 3.88	<0.001
Needle Sources						
Got needles from :						
Pharmacy/drugstore	*No*	78 (56.1)	61 (43.9)	1.00		
	Yes	97 (40.1)	145 (59.9)	1.91	1.25 to 2.92	0.003
Street connections	*No*	121 (59.6)	82 (40.4)	1.00		
	Yes	54 (30.3)	124 (69.7)	3.39	2.21 to 5.18	0.000
Other needle exchanges	*No*	118 (45.7)	140 (54.3)	1.00		
	Yes	57 (46.3)	66 (53.6)	0.98	0.63 to 1.50	0.912
Someone on the street	*No*	156 (47.2)	174 (52.7)	1.00		
	Yes	19 (37.3)	32 (62.7)	1.51	0.82 to 2.77	0.184
Dealer	*No*	154 (47.2)	172 (52.3)	1.00		
	Yes	21 (38.2)	34 (61.8)	1.45	0.81 to 2.60	0.214
Shooting Gallery Owner	*No*	171 (46.8)	194 (53.2)	1.00		
	Yes	4 (25.0)	12 (75.0)	2.64	0.84 to 8.35	0.097
Friends/partners/family	*No*	51 (39.2)	79 (60.8)	1.00		
	Yes	124 (49.4)	127 (50.6)	0.66	0.43 to 1.02	0.060
Sage house	*No*	167 (47.9)	182 (52.1)	1.00		
	Yes	8 (25.0)	24 (75.0)	2.75	1.20 to 6.30	0.016
Sunshine house	*No*	164 (46.2)	191 (53.8)	1.00		
	Yes	11 (42.3)	15 (57.7)	1.17	0.52 to 2.62	0.701
Nine Circles	*No*	156 (45.9)	184 (54.1)	1.00		
	Yes	19 (46.3)	22 (53.7)	0.98	0.51 to 1.88	0.956
Other cities	*No*	159 (44.7)	197 (55.3)	1.00		
	Yes	16 (64.0)	9 (36.0)	0.45	0.20 to 1.05	0.066
Binged in past 6 months	*No*	70 (44.6)	87 (55.4)	1.00		
	Yes	104 (46.6)	119 (53.4)	0.92	0.61 to 1.39	0.693
Drug dependency scale				1.07	1.01 to 1.14	0.020
Network members						
IDU contact	*No*	17 (42.5)	23 (57.5)			
	Yes	158 (46.5)	182 (53.5)	0.85	0.44 to 1.65	0.634
Number of IDU contacts in contactlist	*Scale*			1.04	0.97 to 1.11	0.239
IDU Contact Ethnicity:						
Caucasian	*No*	86 (43.0)	114 (57.0)	1.00		

	Yes	88 (49.4)	90 (50.6)	.77	0.51 to 1.16	0.210
First Nation	*No*	83 (50.6)	81 (49.4)	1.00		
	Yes	91 (42.5)	123 (57.5)	1.39	0.92 to 2.08	0.118
Métis	*No*	136 (47.2)	152 (52.8)	1.00		
	Yes	38 (41.8)	52 (57.8)	1.22	0.76 to 1.97	0.407
Other	*No*	157 (46.0)	184 (54.0)	1.00		
	Yes	17 (45.9)	20 (54.1)	1.00	0.51 to 1.98	0.991
IDU Contact Gender	*Male*	60 (51.7)	56 (48.3)	1.00		
	Female	114 (43.5)	148 (56.5)	1.39	0.90 to 2.16	0.140
IDU Contact Relationship:						
Family	*No*	132 (50.6)	129 (49.4)	1.00		
	Yes	43 (36.1)	76 (63.9)	1.81	1.16 to 2.82	0.009
Lover	*No*	98 (47.3)	109 (52.7)	1.00		
	Yes	60 (45.1)	73 (54.9)	1.09	0.71 to 1.69	0.687
Friend	*No*	42 (42.0)	58 (58.0)	1.00		
	Yes	133 (47.5)	147 (52.5)	0.80	0.50 to 1.27	0.344
Acquaintance	*No*	143 (45.4)	172 (54.6)	1.00		
	Yes	32 (49.2)	33 (50.8)	.86	0.50 to 1.46	0.573
Contact Drug related behaviours						
IDU Contact injects:						
At a hotel	*No*	120 (50.4)	118 (49.6)	1.00		
	Yes	54 (38.0)	88 (62.0)	1.66	1.09 to 2.53	0.019
At a shooting gallery	*No*	137 (47.9)	149 (52.1)	1.00		
	Yes	37 (39.4)	57 (60.6)	1.42	0.89 to 2.28	0.150
At another public place (empty house, hostel, public washroom on the street)	*No*	114 (45.6)	136 (54.4)	1.00		
	Yes	60 (46.2)	70 (53.8)	0.90	0.64 to 1.50	0.918
IDU Contact injects daily	*No*	93 (50.0)	93 (50.0)	1.00		
	Yes	64 (40.3)	95 (59.7)	1.48	0.97 to 2.28	0.070
IDU Contact drug most frequent drug choice						
cocaine	*No*	112 (48.5)	119 (51.5)	1.00		
	Yes	57 (41.0)	82 (59.0)	1.35	0.88 to 2.07	0.162
T and R	*No*	138 (53.5)	120 (46.5)	1.00		
	Yes	31 (27.7)	81 (72.3)	3.00	1.86 to 4.86	<0.001
Morphine	*No*	128 (45.9)	151 (54.1)	1.00		
	Yes	41 (45.1)	50 (54.9)	1.03	0.64 to 1.67	0.891
Heroin	*No*	158 (44.5)	197 (55.5)	1.00		
	Yes	11 (73.3)	4 (26.7)	0.29	0.09 to 0.93	0.038
Crack	*No*	151 (45.6)	180 (54.4)	1.00		
	Yes	18 (46.2)	21 (53.8)	0.97	0.50 to 1.90	0.949
crystalmeth	*No*	146 (42.4)	198 (57.6)	1.00		
	Yes	23 (88.5)	3 (11.5)	0.10	0.03 to 3.26	<0.001
Pooled money to buy drugs or injection equipment (past 6 months)	*No*	49 (44.5)	61 (55.5)	1.00		
	Yes	121 (45.5)	145 (54.5)	0.96	0.62 to 1.50	0.867
Locations IDU contact and individual inject together						
At a private residence	*No*	46 (49.5)	47 (50.5)	1.00		
	Yes	128 (44.6)	159 (55.4)	1.21	0.76 to 1.94	0.414
At a hotel	*No*	146 (47.7)	160 (52.3)	1.00		
	Yes	28 (37.8)	46 (62.2)	1.50	0.89 to 2.52	0.127
At a shooting gallery	*No*	165 (46.7)	188 (53.3)	1.00		
	Yes	9 (33.3)	18 (66.7)	1.76	0.77 to 4.01	0.182
At another public place (empty house, hostel, public washroom on the street)	*No*	137 (44.1)	173 (55.8)	1.00		
	Yes	37 (52.9)	33 (47.1)	0.71	0.42 to 1.19	0.190
Individual and/or IDU contact inject Talwin and Ritalin	*No*	134 (54.5)	112 (45.5)	1.00		
	Yes	41 (30.1)	95 (69.9)	2.77	1.78 to 4.32	<0.001

*OR based on 10 year age difference

† OR based on 5 year age difference

Table 8: Final model of variables significantly associated with HCV outcomes in the study population of IDU in Winnipeg

		Adjusted Odds Ratio	C.I.	*p* value
Age	*OR based on 10 year age difference*	3.39	2.16 to 5.23	<0.001
Age of first injection	*OR based on 5 year difference*	0.70	0.56 to 0.90	<0.001
Injecting in a shooting gallery	*No*	1.00		
	Yes	3.67	1.49 to 9.02	0.005
Ever having shared a used needle	*No*	1.00		
	Yes	3.87	2.15 to 6.98	<0.001
Ever having sold drugs	*No*	1.00		
	Yes	0.48	0.27 to 0.88	0.016
Ever having injected someone else as a service (i.e. in exchange for money, drugs or goods)	*No*	1.00		
	Yes	2.75	1.31 to 5.77	0.007
Obtaining needles from Street Connections	*No*	1.00		
	Yes	2.38	1.33 to 4.27	0.007
Talwin and Ritalin use (individual and/or by an IDU in their network)	*No*	1.00		
	Yes	2.57	1.37 to 4.84	0.003

Table 9: Interaction effects of syringe sharing on Talwin and Ritalin use in outcomes of HCV

		Reported injection with a used syringe	
		Yes	No
Linked with Talwin and Ritalin use	Yes	1.36 (0.20 to 2.32)	1.00
	No	7.03 (5.67 to 8.30)	1.00

Table 10: HCV outcomes by interacting variables

		Reported injection with a used syringe	
		Yes	No
Linked with Talwin and Ritalin use	Yes	57 HCV + /14 HCV -	30 HCV + /17 HCV -
	No	82 HCV+ /45 HCV -	22 HCV + /75 HCV -

4.4 Results for Objective 2: Determine which sociodemographic and sociobehavioural variables are correlated with infection by either HCV genotypes 1a or 3a

4.4.1 Univariate and multivariate results of Objective 2

Of the 207 HCV positive specimens, 16 (7.7%) samples were insufficient in volume after diagnosis for sequencing and 50 (24.2%) samples had undetectable PCR products. Separate consent was also required for the genotyping and two individuals refused consent for this part of the study.

Of the HCV positive specimens 139 (66.5%) were successfully genotyped. Of HCV positive study participants, the most prevalent genotypes were 1a and 3a (Table 11). Small numbers of 1b, 2a and 2b were also present in the typed samples. This analysis used data only for those individuals diagnosed with genotypes 1a or 3a, as only these two genotypes were common enough in the study population to allow analysis. Univariate and multivariate results for Objective 2 are presented in Table 12. Variables from Objective 1 that are not present in Objective 2 were removed because of a low number of observations during univariate analysis.

After univariate analysis, injecting on the street, not using barbiturates, pooling money to buy drugs or injection equipment with a network IDU member, younger age, and injecting preferred drug choice, were all associated with a greater likelihood of being infected with genotype 3a (Table 12).

After multivariate analysis, genotype 3a was significantly associated with younger age and injection on the street (Table 13). Significant interactions were tested, but none were found to affect the final model. The Hosmer-Lemeshow goodness of fit for the final model was 0.64.

Table 11: Genotype distribution of sequenced HCV positive specimens

	n	% of total genotyped specimens
1a	82	59.0
3a	47	33.8
1b	5	3.6
2a	3	2.2
2b	2	1.4
Total	139	100.00

Table 12: Univariate analysis of genotype outcome

		Genotype 1a N (%)	Genotype 3a N (%)	Odds Ratio	95% Confidence Interval	p value
Total subjects		82 (63.57)	47 (36.43)			
Demographics						
Age*	*Mean (SD)*	39.6	35.6	0.53	0.31 to 0.81	0.011
Gender						
	Male	48 (63.2)	28 (36.8)	1.00		
	Female	34 (64.2)	19 (35.8)	0.96	0.46 to 1.98	0.908
Ethnicity						
	Caucasian	20 (60.1)	13 (39.4)	1.00		
	First Nation (treaty/non-treaty)	45 (65.2)	24 (34.8)	0.82	0.35 to 1.93	0.651
	Métis	15 (60.0)	10 (40.0)	1.02	0.35 to 2.97	0.963
Birthplace						
	Winnipeg	33 (64.7)	18 (35.3)	1.00		
	rural Manitoba	31 (67.4)	15 (32.6)	0.89	0.38 to 2.07	0.782
	Canada (outside Manitoba)	17 (58.6)	12 (41.4)	0.47	0.50 to 3.32	0.591
Primary source of income						
	Regular work	11 (55.0)	9 (45.0)	1.00		
	Financial support (Government/friends or family)	66 (66.7)	33 (33.3)	0.61	0.23 to 1.63	0.321
	Illegal activities	5 (50.0)	5 (50.0)	1.22	0.26 to 5.75	0.799
Other sources of income over the last year:						
Regular work	*No*	50 (62.5)	30 (37.5)	1.00		
	Yes	32 (65.3)	17 (34.7)	0.89	0.42 to 1.87	0.749
Welfare	*No*	66 (63.5)	38 (36.5)	1.00		
	Yes	16 (64.0)	9 (36.0)	0.98	0.39 to 2.43	0.960
Money from family or friends	*No*	43 (63.2)	25 (36.8)	1.00		
	Yes	39 (63.9)	22 (36.1)	0.97	0.47 to 2.00	0.935
Sex trade	*No*	65 (66.3)	33 (33.7)	1.00		
	Yes	17 (54.8)	14 (45.2)	1.62	0.71 to 3.72	0.249
Dealing drugs	*No*	37 (57.0)	28 (43.1)	1.00		
	Yes	45 (70.3)	19 (29.7)	0.56	0.27 to 1.17	0.116
Panhandling	*No*	69 (65.7)	36 (34.3)	1.00		
	Yes	13 (54.2)	11 (45.8)	1.62	0.66 to 4.01	0.291
Boost or Stealing	*No*	45 (63.4)	26 (36.6)	1.00		
	Yes	37 (63.8)	21 (36.2)	0.98	0.47 to 2.03	0.962
Number of places lived in the last year	*Scale*			1.00	0.82 to 1.84	0.88
Moved to Winnipeg in the past year	*No*	62 (63.3)	36 (36.7)	1.00		
	Yes	20 (64.5)	11 (35.5)	0.95	0.41 to 2.21	0.900
Drug use related behaviours						
Age of first injection†	*Mean (SD)*	20.4	20.1	0.95	0.73 to 1.28	0.860
Non-injection drugs:						
Alcohol	*No*	10 (66.7)	5 (33.3)	1.00		
	Yes	72 (63.2)	42 (36.8)	1.17	0.37 to 3.66	0.792
Painkillers	*No*	43 (61.4)	27 (38.6)	1.00		
	Yes	39 (66.1)	20 (33.9)	0.82	0.40 to 1.69	0.584
Amphetamines	*No*	65 (62.5)	39 (37.5)	1.00		
	Yes	17 (68.0)	8(32.0	0.78	0.31 to 2.00	0.609
Barbiturates	*No*	32 (51.6)	30 (48.4)	1.00		
	Yes	50 (74.6)	17 (25.4)	0.36	0.17 to 0.78	0.007
Cocaine	*No*	23 (57.5)	17 (42.5)	1.00		
	Yes	59 (66.3)	30 (33.7)	0.69	0.32 to 1.49	0.339
Crack	*No*	14 (56.0)	11 (44.0)	1.00		
	Yes	68 (65.4)	36 (34.6)	0.67	0.28 to 1.65	0.383

Demerol/morphine/opium	*No*	43 (59.7)	29 (40.3)	1.00		
	Yes	39 (68.4)	18 (31.6)	0.68	0.33 to 1.43	0.310
Downers/Tranquilizers	*No*	24 (61.5)	15 (38.5)	1.00		
	Yes	58 (64.4)	32 (35.5)	0.88	0.40 to 1.92	0.754
Gasoline/Solvents	*No*	70 (67.3)	34 (32.7)	1.00		
	Yes	12 (48.0)	13 (52.0)	2.23	0.91 to 5.49	0.073
Marijuana	*No*	21 (65.6)	11 (34.4)	1.00		
	Yes	61 (62.9)	36 (37.1)	1.13	0.49 to 2.61	0.781
Tylenol #3	*No*	31 (67.4)	15 (32.6)	1.00		
	Yes	51 (61.4)	32 (38.5)	1.30	0.60 to 2.78	0.503
Mushrooms	*No*	67 (62.0)	41 (38.0)	1.00		
	Yes	15 (71.4)	6 (28.6)	0.65	0.23 to 1.83	0.415
Methadone (prescribed or unprescribed)	*No*	52 (59.8)	35 (40.2)	1.00		
	Yes	30 (71.4)	12 (28.6)	0.59	0.27 to 1.33	0.199
Crystal meth	*No*	72 (63.7)	41 (36.3)	1.00		
	Yes	10 (62.5)	6 (37.5)	1.05	0.36 to 3.12	0.925
Other	*No*	67 (62.6)	40 (37.4)	1.00		
	Yes	15 (68.2)	7 (31.8)	0.78	0.29 to 2.09	0.623
If drug injected is same as preferred drug	*No*	32 (76.2)	10 (23.8)	1.00		
	Yes	50 (57.5)	37 (42.5)	2.37	1.02 to 5.51	0.040
Drugs injected in last 6 months:						
Cocaine	*No*	23 (57.5)	17 (42.5)	1.00		
	Yes	59 (66.3)	30 (33.7)	0.69	0.32 to 1.49	0.339
Talwin and Ritalin	*No*	52 (65.8)	21 (34.2)	1.00		
	Yes	30 (60.0)	20 (40.0)	1.28	0.61 to 2.68	0.505
Morphine	*No*	53 (62.4)	32 (37.6)	1.00		
	Yes	29 (65.9)	15 (34.1)	0.86	0.40 to 1.84	0.692
Crack/Rock cocaine	*No*	62 (63.3)	36 (36.7)	1.00		
	Yes	20 (64.5)	11 (35.5)	0.95	0.41 to 2.21	0.900
How often shot up in the past month	*Not at all,*	22 (64.7)	12 (35.3)	1.00		
	Once in a while, not every week	16 (55.2)	13 (44.8)	1.49	0.53 to 4.16	0.444
	Regularly, once or twice a week	12 (70.6)	5 (29.4)	0.76	0.21 to 2.72	0.678
	Regularly, three or more times a week	19 (73.0)	7 (26.9)	0.68	0.22 to 2.09	0.493
	Everyday	13 (56.5)	10 (43.5)	1.41	0.47 to 4.22	0.537
Injection sites:						
Empty house	*No*	71 (64.0)	40 (36.0)	1.00		
	Yes	11 (61.1)	7 (38.9)	1.13	0.40 to 3.16	0.816
Hotel	*No*	50 (67.6)	24 (32.4)	1.00		
	Yes	32 (58.2)	23 (41.8)	1.50	0.72 to 3.11	0.275
Shooting gallery	*No*	68 (66.0)	35 (34.0)	1.00		
	Yes	14 (53.8)	12 (46.2)	1.67	0.69 to 4.02	0.251
Rooming/Boarding house	*No*	53 (62.4)	32 (37.6)	1.00		
	Yes	29 (65.9)	15 (34.1)	0.86	0.40 to 1.84	0.692
On the street	*No*	65 (72.2)	25 (27.8)	1.00		
	Yes	17 (43.6)	22 (56.4)	3.36	1.49 to 7.62	0.002
Vehicle	*No*	63 (67.7)	30 (32.3)	1.00		
	Yes	19 (52.8)	17 (47.2)	1.88	0.85 to 4.17	0.115
Public Washroom	*No*	68 (65.4)	36 (34.6)	1.00		
	Yes	14 (56.0)	11 (44.0)	1.48	0.61 to 3.63	0.383
Used someone else's cooker, rinse water or cotton (past 6 months)	*Never*	51 (64.6)	28 (35.4)	1.00		
	Ever	26 (57.8)	19 (42.2)	1.33	0.63 to 2.83	0.456
Ever used someone else's needle	*No*	22 (68.8)	10 (31.2)	1.00		
	Yes	55 (61.1)	35 (38.9)	1.40	0.59 to 3.32	0.444
Sold drugs	*No*	41 (57.7)	30 (42.3)	1.00		
	Yes	40 (71.4)	16 (28.6)	0.55	0.26 to 1.17	0.113

How many times injected someone else for drugs money or other goods	*Never*	56 (65.1)	30 (34.9)	1.00		
	Ever	26 (60.5)	17 (39.5)	1.22	0.57 to 2.61	0.606
How many times injected someone else with drugs as a favour	*Never*	42 (63.4)	24 (36.4)	1.00		
	Ever	39 (62.9)	23 (37.1)	1.03	0.50 to 2.12	0.932
Needle Sources						
Got needles from :						
Pharmacy/drugstore	*No*	27 (67.5)	13 (32.5)	1.00		
	Yes	55 (61.8)	34 (38.2)	1.28	0.58 to 2.83	0.535
Street connections	*No*	33 (63.5)	19 (36.5)	1.00		
	Yes	49 (63.4)	28 (36.4)	0.99	0.48 to 2.07	0.984
Other needle exchanges	*No*	56 (63.6)	32 (36.4)	1.00		
	Yes	26 (63.4)	15 (36.6)	1.01	0.47 to 2.19	0.981
Someone on the street	*No*	70 (63.6)	40 (36.4)	1.00		
	Yes	12 (63.2)	7 (36.8)	1.02	0.37 to 2.81	0.968
Dealer	*No*	71 (64.0)	40 (36.0)	1.00		
	Yes	11 (61.1)	7 (38.9)	1.13	0.40 to 3.16	0.816
Friends/partners/family	*No*	31 (64.6)	17 (35.4)	1.00		
	Yes	51 (63.0)	30 (37.0)	1.07	0.51 to 2.26	0.854
Sage house	*No*	74 (64.9)	40 (35.1)	1.00		
	Yes	8 (53.3)	7 (46.7)	1,62	0.54 to 4.83	0.383
Binged in past 6 months	*No*	37 (63.8)	21 (36.2)	1.00		
	Yes	45 (63.4)	26 (36.6)	1.02	0.49 to 2.10	0.962
Depression scale				0.94	0.87 to 1.01	0.076
Egocentric network data						
IDU contact	*No*					
	Yes					
Number of IDU contacts in contactlist	*Scale*			0.97	0.87 to 1.07	0.538
IDU Contact Ethnicity:						
Caucasian	*No*	46 (60.5)	30 (39.5)	1.00		
	Yes	34 (66.7)	17 (33.3)	0.77	0.36 to 1.62	0.484
First Nation	*No*	36 (63.2)	21 (36.8)	1.00		
	Yes	44 (62.9)	26 (37.1)	1.01	0.49 to 2.10	0.972
Métis	*No*	63 (65.6)	33 (34.4)	1.00		
	Yes	17 (54.8)	14 (45.2)	1.57	0.69 to 3.61	0.281
IDU Contact Gender	*Male*	22 (59.4)	15 (40.5)	1.00		
	Female	59 (64.8)	32 (35.2)	0.80	0.36 to 1.75	0.569
IDU Contact Relationship:						
Family	*No*	53 (64.6)	29 (35.4)	1.00		
	Yes	28 (60.9)	18 (39.1)	1.17	0.55 to 2.48	0.673
Lover	*No*	45 (64.3)	25 (35.7)	1.00		
	Yes	22 (52.4)	20 (47.6)	1.64	0.74 to 3.60	0.215
Friend	*No*	24 (61.5)	15 (38.5)	1.00		
	Yes	57 (64.0)	32 (36.0)	0.90	0.41 to 1.96	0.787
Acquaintance	*No*	67 (62.0)	41 (38.0)	1.00		
	Yes	14 (70.0)	6 (30.0)	0.70	0.25 to 1.98	0.499
Risk network data						
IDU Contact injects:						
At a hotel	*No*	51 (63.0)	30 (37.0)	1.00		
	Yes	31 (64.6)	17 (35.4)	0.93	0.44 to 1.97	0.854
At a shooting gallery	*No*	61 (63.5)	35 (36.5)	1.00		
	Yes	21 (63.6)	12 (36.4)	1.00	0.44 to 2.27	0.992
At another public place (empty house, hostel, public washroom on the street)	*No*	59 (67.0)	29 (33.0)	1.00		
	Yes	23 (56.1)	18 (43.9)	1.59	0.74 to 3.41	0.230
IDU Contact injects daily	*No*	42 (63.6)	24 (36.4)	1.00		
	Yes	33 (67.3)	16 (32.7)	0.85	0.39 to 1.86	0.681
IDU Contact drug most frequent drug choice						

cocaine	*No*	39 (58.2)	28 (41.8)	1.00		
	Yes	38 (66.7)	19 (33.3)	0.70	0.33 to 1.46	0.335
T and R	*No*	51 (63.0)	30 (37.0)	1.00		
	Yes	26 (60.5)	17 (39.5)	1.11	0.52 to 2.38	0.786
Morphine	*No*	60 (62.5)	36 (37.5)	1.00		
	Yes	17 (60.7)	11 (39.3)	1.08	0.45 to 2.57	0.865
Crack	*No*	69 (62.2)	42 (37.8)	1.00		
	Yes	8 (61.5)	5 (38.5)	1.03	0.31 to 3.36	0.965
Pooled money to buy drugs or injection equipment (past 6 months)	*No*	34 (77.3)	10 (22.7)	1.00		
	Yes	48 (56.5)	37 (43.5)	2.62	1.15 to 5.98	0.022
Locations IDU contact and individual inject together						
At a private residence	*No*	22 (66.7)	11 (33.3)	1.00		
	Yes	60 (62.5)	36 (37.5)	1.20	0.52 to 2.77	0.669
At a hotel	*No*	65 (63.7)	37 (36.3)	1.00		
	Yes	17 (63.0)	10 (37.0)	1.03	0.43 to 2.50	0.942
At another public place (empty house, hostel, public washroom on the street)	*No*	70 (66.0)	36 (34.0)	1.00		
	Yes	12 (52.2)	11 (47.8)	1.78	0.71 to 4.48	0.212
Individual and/or IDU contact inject Talwin and Ritalin	*No*	49 (65.3)	26 (34.7)	1.00		
	Yes	33 (61.1)	21 (38.9)	1.20	0.58 to 2.48	0.623

*OR based on 10 year age difference

† OR based on 5 year age difference

Table 13: Final model of variables significantly associated with genotype outcomes in the study population of IDU in Winnipeg

		Adjusted Odds Ratio	C.I.	*p* value
Age	*OR based on 10 year age difference*	0.59	0.37 to 0.95	0.029
Injecting on the street	*No*	1.00		
	Yes	3.05	1.37 to 6.80	0.006

Note: The final model was not adjusted for gender or ethnicity as neither significantly altered the overall goodness of fit for the model

4.5 Results for Objective 3: Determine whether correlations between social variables and HCV subtypes (i.e. below the genotype level) exist.

All 82 genotype 1a and the 47 genotype 3a from the previous analysis were entered into AI analysis (129 study participants).

Figure 6 shows the complete HCV genotype dataset with one test sequence per study participant along with reference sequences. Phylogenetic trees were also constructed using only the sequences for the 1a and 3a genotype. Figure 7 shows a neighbour joining tree for genotype 1a and genotype 3a. Figure 8 and Figure 9 are enlarged diagrams of the 1a and 3a segments of the neighbour joining tree of Figure 7, where the numerical tags along the length of the phylogenetic tree indicate the non-nominal study participant numbers.

Variables with AI values of <1.0 are summarized in Table 13. Percentile ranges of test sequences and control sequences were compared for overlap (i.e. does significant segregation exist for a given variable). Significant segregation was identified for the following variables: injected on the street (0.89), injection at a hotel (0.84), injection in a public washroom (0.84), income from sextrade (0.89), moved to Winnipeg within the last year (0.84) and being associated with Talwin and Ritalin use (0.89). Although certain variables have significant AI values, these differences would be considered moderate.

Figure 10 and Figure 11 show the distribution of these respective variables within the phylogenetic tree. The "modest" segregation manifests with each variable being scattered throughout the tree and a moderate amount of clustering in only one or two areas of the tree.

Figure 6: Unrooted dendrogram of HCV sequences in Winnipeg injection drug user study sample with reference HCV strains (Bionumerics, Applied Maths) ref=reference sequences

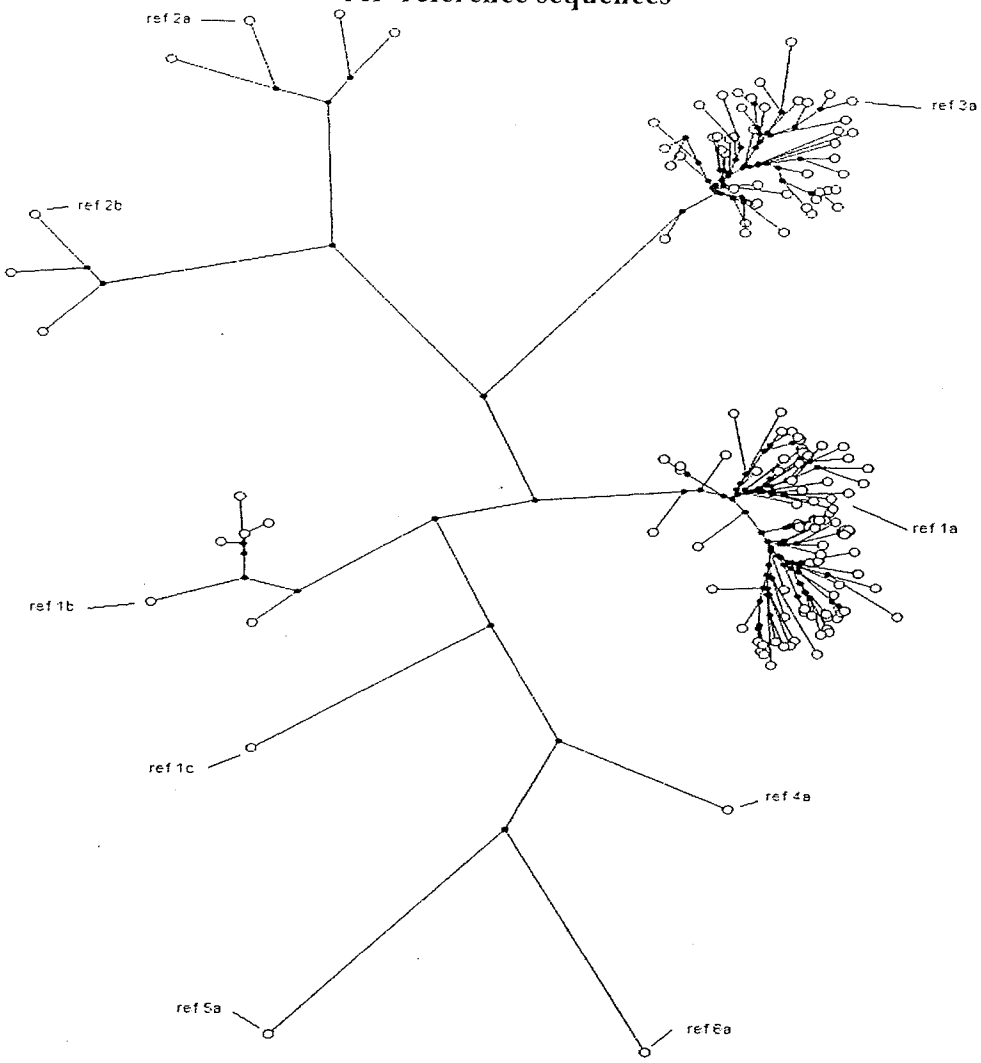


Figure 7. Neighbour joining phylogenetic tree based on Tamura-Nei substitution model constructed of HCV sequences amplified from injection drug users in Winnipeg

Note: study participant numbers and numerical tags are magnified in Figures 8 and 9

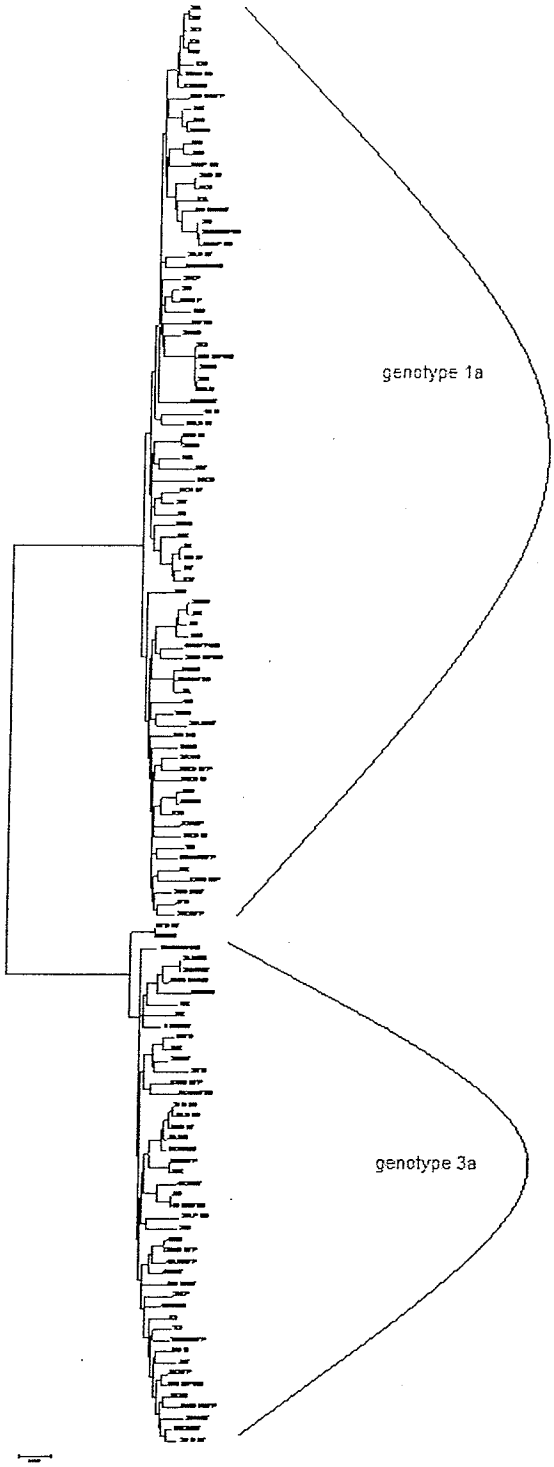


Figure 8: Neighbour joining phylogenetic tree based on Tamura-Nei substitution model of genotype 1a sequences (MEGA)

number= numeric study participant identifier

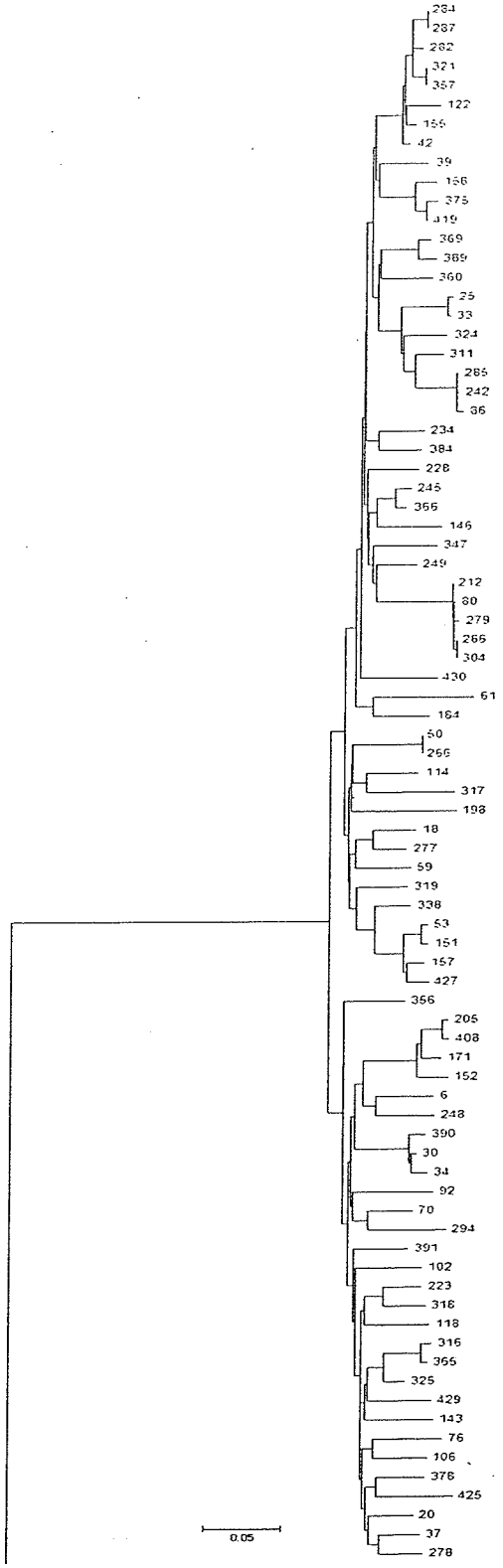


Figure 9: Neighbour-Joining phylogenetic tree for genotype 3a (MEGA)

number= numeric study participant identifier

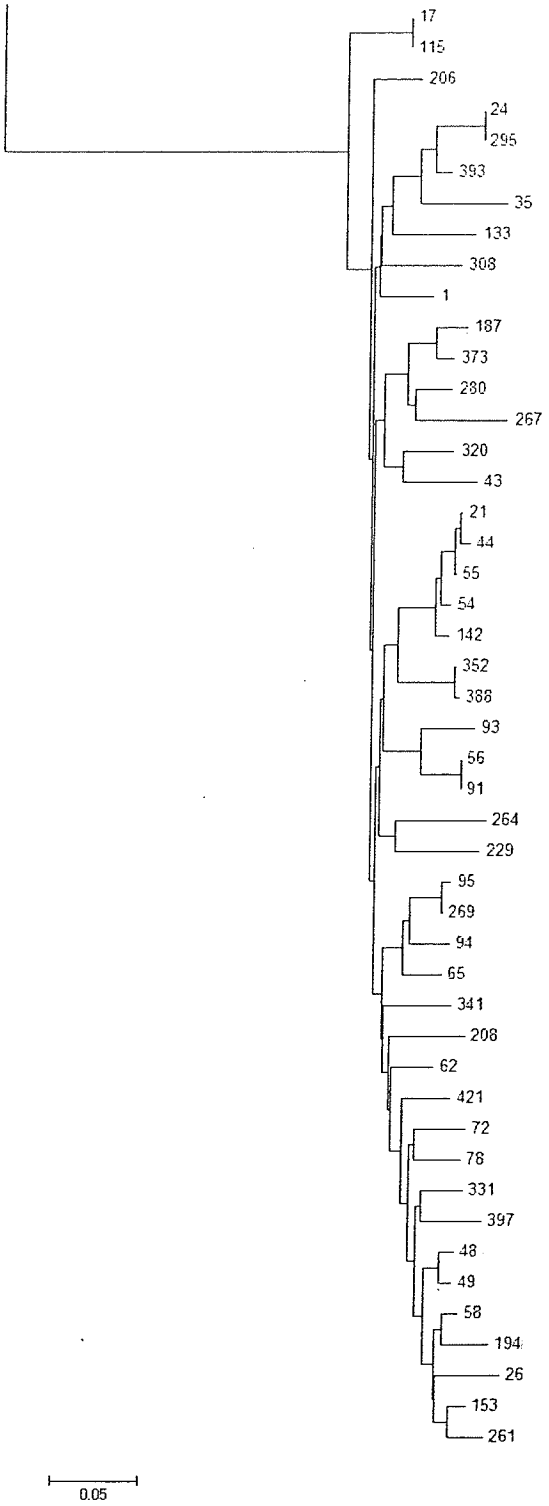


Table 14: AI values and percentile ranges for demographics and AI values under 1.0

	AI		Test sequences percentile range		Control percentile range	Upper percentile of test sequence is less than lower sequence of control sequences
Age	0.94	15.93	14.7-17.3	17.0	16.2-17.8	N.s.
Ethnicity	0.95	19.6	18.4 - 20.5	20.5	19.8 - 21.2	N.s.
Hotel	0.84	14.0	12.7 - 15.1	16.7	15.8 - 17.3	Significant
Shooting Gallery	0.90	9.9	8.9 - 10.8	10.9	10.3 - 11.5	N.s.
Street injection	0.89	12.7	11.6-13.7	14.3	13.6-15.0	N.s.
Public washroom	0.84	8.9	7.9 - 9.9	10.6	10.1 - 11.1	Significant
Sextrade as primary income	0.89	11.1	9.9 - 12.1	12.4	11.9 - 13.1	N.s.
Sextrade as primary or secondary income	0.87	11.1	10.0 - 12.0	12.8	12.2 - 13.4	Significant
Moved to Winnipeg	0.84	10.3	9.3 - 11.4	12.3	11.7 - 12.9	Significant
Individual and network Talwin and Ritalin use	0.89	14.7	13.6 - 15.8	16.5	15.8 - 17.2	Borderline
Injected with an IDU contact in a public	0.90	8.9	8.0 - 9.8	10.0	9.5-10.6	N.s.

Figure 10: Neighbour-Joining Tree based on Tamura-Nei substitution model with characteristics for genotype 1a (MEGA)

numbers = numeric study participant identifier; H=injected at a Hotel, M=Moved to Winnipeg, SE= income from sextrade,, P=injects in a public washroom

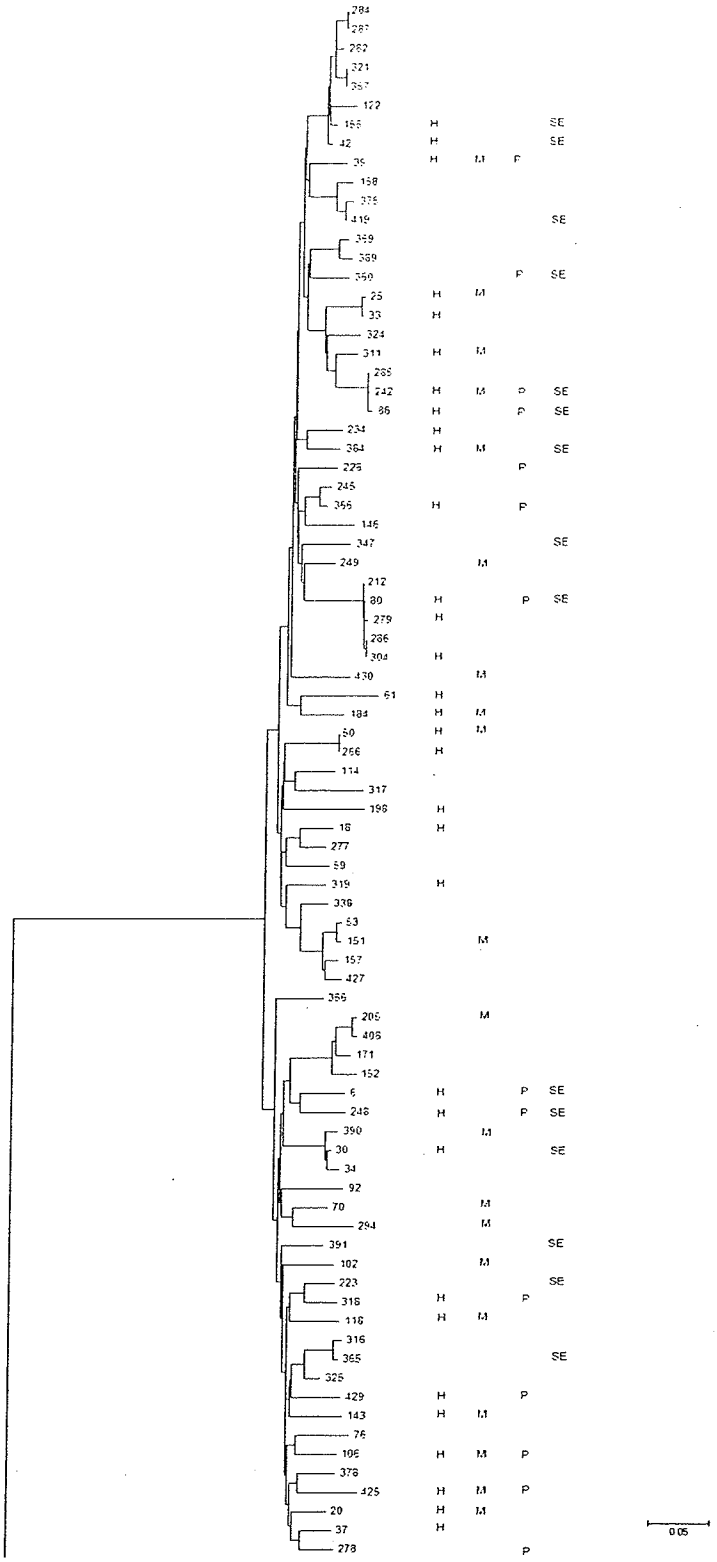
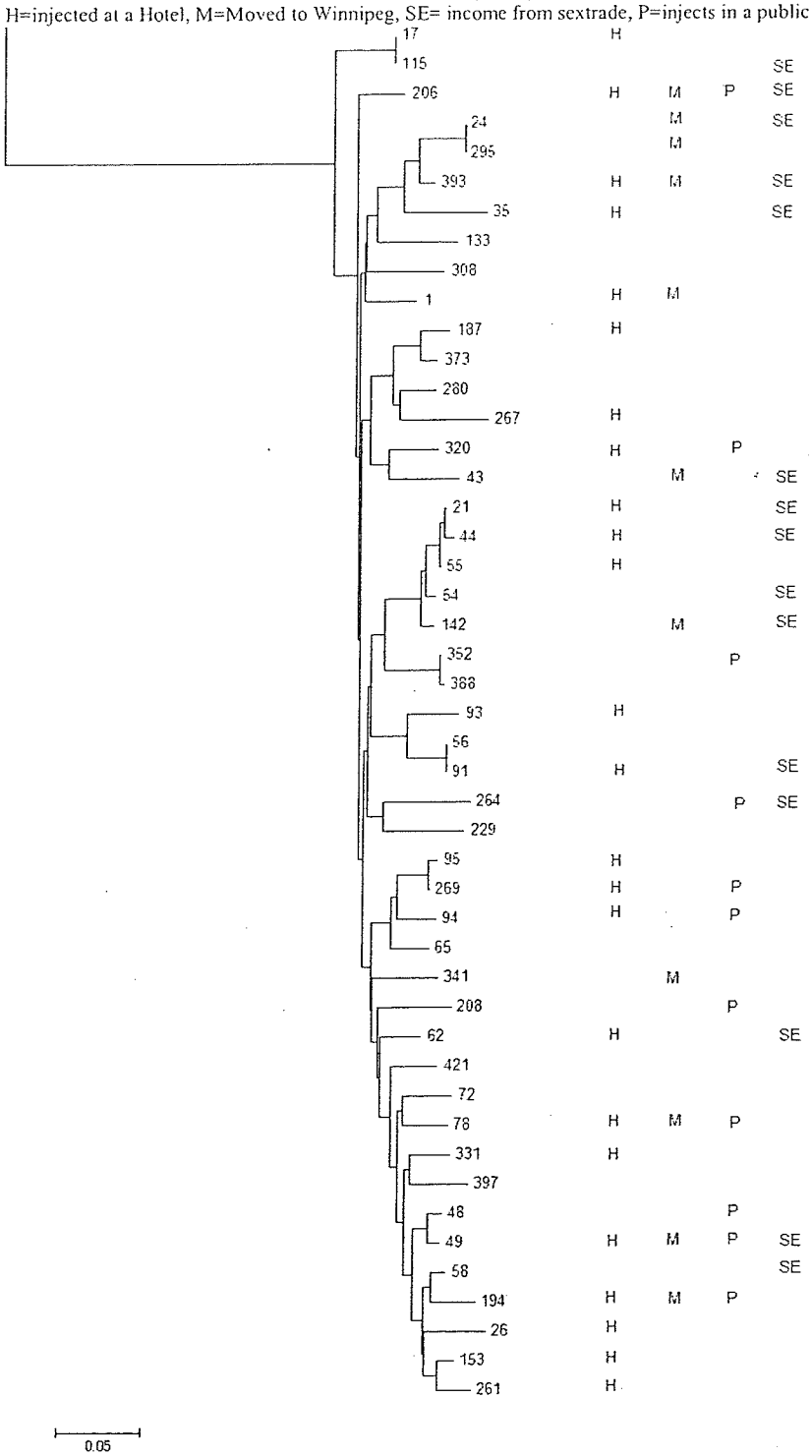


Figure 11: Neighbour Joining tree based on Tamura-Nei substitution model with characterisitics for 3a sequences (MEGA)

numbers = numeric study participant identifier;
H=injected at a Hotel, M=Moved to Winnipeg, SE= income from sextrade, P=injects in a public washroom



Chapter 5: Discussion

5.1 Discussion of Objective 1: Correlate the seroprevalence of HCV with individual and social network variables.

Prior to this study, the prevalence of HCV amongst IDU in Winnipeg was unknown. Elsewhere in Canada, the HCV prevalence amongst IDU has been shown to be as high as 88% [91, 92]. The HCV prevalence in our study population is significantly lower than the Canadian average of HCV amongst IDU (estimated at around 80%) [91]. The lower prevalence we found in this study population could be real or could be artifactual due to differences in study design or sample size. Nonetheless, due to rapid transmissibility of HCV amongst IDU, the potential for rapid spread of HCV within Winnipeg IDU is considerable.

In our population, several individual characteristics were correlated with HCV positivity. The association of many of these variables is not unexpected, and has been reported in other studies [7, 88]. Self-reported injection with used syringes represents a direct route of transmission for HCV and other bloodborne pathogens [93]. Also, those IDU with long injection careers - in our study, both older age and initiation of injection at a young age were correlated with the length of an individual's injection career - reflects an ever increasing likelihood of potential exposure to a contaminated syringe or equipment [7]. Injection at a shooting gallery has also been linked to an increased likelihood of purposefully or accidentally using contaminated equipment and an HCV positive outcome [10, 94]. The latter correlation may also be a proxy measure for high risk, largely anonymous, networks of IDU which may provide bridging opportunities for pathogen transmission among individuals who may not know each other.

Injecting someone as a service has been correlated to the sharing of syringes as part of this interaction (the injector may first use a needle and then inject someone else with that same needle) [95]. In our study, a correlation between this variable and HCV positivity was significant even after controlling for syringe sharing. Recall of injecting someone as a service may be more precise than recall of syringe sharing, which would make this variable a more concise marker for high risk behaviour associated with HCV transmission. However, details of the local behavioural patterns associated with this practice in our population and its correlation with HCV positivity would need to be confirmed by additional research specifically designed to address this question.

An association between needle exchange use and high risk injection drug use was identified in our analysis. Similar results have been observed in other jurisdictions [36, 96]. It has been argued that the highest-risk IDU (and hence those IDU most likely to be infected with a bloodborne pathogen) are those most likely to use a needle exchange [97]. This is likely the origin of the correlation we found in our dataset, however, as above, further research would be needed to explore the link between needle exchanges and infection status for our local area.

Non-injecting sources were surveyed in this questionnaire, but inclusion in this study required injection use of drugs within the past six months. A small amount of transmission is thought to occur from sexual transmission, tattooing and needle-stick injuries, intranasal cocaine and crack smoking possible transmission routes [98]. At the moment, crystal meth (methamphetamine) users are univariately associated with significantly decreased outcomes of HCV. However, the potential for this group to

become infected exists and as a preventative measure, this potential route of transmission should be addressed.

Crystal meth use, both smoking and injecting has recently been introduced to the Winnipeg drug scene; in the WIDE (1999) study it was not a drug choice commonly mentioned [34]. Although at the univariate-level, crystal meth users were statistically less likely to be HCV positive, the implications of this relationship are not fully understood. Individuals who smoke crystal meth in this study population, do not appear involved in the core HCV positive injection drug use scene. Nevertheless, two potential changes could impact crystal meth users in this population: the first is the potential transition from smoking to injection of crystal meth, and secondly that the burns and cuts resulting from smoking crystal meth could become a route of HCV transmission. Studies have shown that transition from non-injection to injection use of drugs is likely in high risk groups [99-101]. Given that those reporting smoking crystal meth in this population are already currently or have previously injected drugs, the risk for transition appears elevated. Secondly, the survey questionnaire indirectly uncovered the occurrence of burns from smoking crystal meth when inquiring about sores and burns from crack smoking. Previously crack smoking has been associated with HCV transmission due to lesions in the mouth [10]. Similarly, the potential introduction of HCV into the segment of the IDU population smoking crystal meth exists even if at this time these individuals are less likely to be HCV positive.

Talwin and Ritalin use appears to have increased since WIDE, with this drug combination being the most frequently reported injection drug in this study, after cocaine. The short term effects of Talwin and Ritalin have been compared to the effects of heroin

mixed with cocaine; although Talwin and Ritalin is considerably lower in cost and can be obtained legally and on the street. Use of Talwin and Ritalin was associated with HCV positivity, whereas no association was identified with the use of other drug types. This pattern may reflect the existence of a high risk network formed by IDU who use Talwin and Ritalin. Distinct types of networks formed by Talwin and Ritalin users have not been studied in terms of HCV transmission as these drugs are rarely used in most jurisdictions. Krekulova *et al.* (2001) has found, however, that distinct transmission networks do form amongst users of specific drugs [30]. Similar observations have been made by Crofts *et al.* (1994) based on the general distribution pattern of bloodborne pathogens amongst Australian IDU [31].

Notably, Talwin and Ritalin use interacted with self-reported injection with a used syringe. For IDU not linked to Talwin and Ritalin use, the expected correlation between HCV positivity and injection with a used syringe was observed. This relationship was absent from the Talwin and Ritalin group, such that those not reporting injections with a used syringe were as likely to have experienced HCV infection as those reporting this behaviour. This characteristic may be due to a number of factors including inaccurate recall of behaviours or unique behavioural attributes associated with the use of these drugs. Talwin and Ritalin is prepared at room temperature with no heat (indirectly aiding pathogen survival) and is prepared communally. Users typically will draw up drug using their own syringe but a common filter. If any one syringe is contaminated, pathogens could be easily transmitted to the drug mixture and other user's syringes (Margaret Ormond personal communication).

Regardless of the underlying reason, there is a high risk for HCV acquisition associated with Talwin and Ritalin use in our population. This group, and analogous high risk groups in other locales, may form a core group for transmission of certain pathogens and may be important as key points of intervention for public health programmes aimed at reducing the incidence of infectious diseases amongst IDU. Although Talwin and Ritalin use is unique to certain jurisdictions, primarily in Western Canada, and has not been fully described in the literature, this analysis underlines the importance of networks formed within the IDU population based on drug choices. Furthermore it suggests that network drug choices are extremely important in combination with individual behaviours in determining risk of HCV acquisition.

The link between Talwin and Ritalin use and HCV positivity could also partially explain the protective effect of selling drugs in our population. Although elsewhere the selling of drugs has been associated with higher risk behaviours [23], in our study population this variable may represent IDU with relatively infrequent exposure to HCV; selling drugs may be a proxy measure for other behaviours that may lead to decreased high risk behaviours. Preliminary exploratory analysis of our dataset suggests that drug sellers in Winnipeg are less likely to be Talwin and Ritalin users (data not shown). The apparently protective effect of some behaviours may therefore reflect partial isolation from the highest risk transmission networks where exposure to bloodborne pathogens are common. An understanding of the local transmission networks that exist in an area is critical for a full understanding and interpretation of the effects of specific variables.

In the context of prevention of HCV transmission amongst IDU, harm reduction has had limited impact [13, 36, 91] . Our findings suggest factors other than self-reported

use of potentially contaminated needles are relevant in identifying individuals at risk of HCV acquisition. Although Talwin and Ritalin use and other related behaviours, may differ between IDU populations, the results of our study underline the importance of drug choice, both individual and at the network-level, as a mediator of risk of HCV acquisition amongst individuals who do not report use of contaminated needles.

5.2 Discussion of Objective 2: Determine which sociodemographic and sociobehavioural variables are correlated with infection by either HCV genotypes 1a or 3a.

A total of 50 (24.2%) HCV antibody positive specimens did not produce PCR results, even after using primers for the conserved region of the HCV genome. This discrepancy could be explained by viral clearance and low viral loads; an estimated 20% of HCV infections are cleared by host without treatment [102]. The immune response to HCV is not well understood, and is being studied in other parts of the overall study as discussed in the introduction of this thesis. Published studies have demonstrated a range of amplifiable HCV RNA from 54% to 86 % [103]. The genotyped HCV in our study of greater than 70% falls within this range.

The relative proportion of HCV genotypes circulating in this study population is consistent with results in other IDU populations. Genotypes 3a and 1a are typically associated with IDU in other locales [21, 27, 62, 104, 105]. Elsewhere genotype 1b has been associated with HCV acquired through blood transfusions as well as IDU populations [21, 27, 53]. Genotype 1b is a prominent genotype in both IDU and non-IDU HCV cases in Europe [30, 106]. In our study population, genotypes 1b, 2a and 2b genotypes were rare and the variables associated with transmission of these genotypes could not accurately be investigated.

In our study population, genotypes 3a and 1a were distinguished by two characteristics: age and injection on the street (Table 12). For a ten year age difference, younger participants were 1.67 times more likely to have genotype 3a and individuals who reported injecting on the street were more likely to have genotype 3a (OR 3.05)when compared to study participants carrying genotype 1a.

Studies in other areas have also found genotype 3 to be associated with younger age. Age has been identified as an important delineator of network boundaries (i.e. concordant mixing by age is a protective factor for avoiding HIV transmission, while discordant mixing acts as a bridge for transmission – see page 13) [19]. Segregation of genotype by age in our site also suggests that age mixing amongst Winnipeg IDU is concordant and may define relatively distinct transmission networks for bloodborne (and possibly other) pathogens.

The geographic settings at which injection drug use occurs (drug settings), such as shooting galleries, have been associated with a greater likelihood of sharing needles and contaminated injection paraphernalia [107, 108]. As such, specific drug settings could act as focal points for the formation of transmission networks. Although "injecting on the street" represents an undefined physical space, this variable could represent a drug setting frequented by a distinct type of user. For example, street injection in Winnipeg could be associated with mobile, traveling youth or other socioeconomically distinct subgroups within the larger IDU population. Their behaviours may lead to relatively little mixing with other IDU, again contributing to the creation of distinct transmission networks. Segregation of IDU along socioeconomic lines has been identified in other areas [107, 109-111]. In Hungary, IDU could be classified as either socially integrated, with regular employment and who sniffed cocaine, or socially marginalized, without regular employment, and who sniffed glue [111]. Here, social integration vs. social marginalization was viewed as variables that defined separate networks within the larger IDU population. Similar factors could be operating within Winnipeg where division of IDU occur along socioeconomic lines.

From a treatment perspective, the genotype distribution in a population is crucial to ensuring effective control, given that different HCV genotypes respond differently to treatment. HCV genotype 1 requires a longer treatment period and only has a probability of sustained viral clearance of 40-46% compared to genotype 3 with a clearance probability of 75-82% [112-114]. Since treatment success is higher for genotype 3a, the possibility exists that, over time, selection for genotype 1a could occur. Despite this possibility it appears that genotype 3a is emerging to become the predominant type amongst IDU [115]. In our setting, if genotype 3a is emerging in Winnipeg, as noted above, it appears to be occurring amongst younger injectors and those who inject on the street. To our knowledge, the emergence of 3a is not well understood, and whether this is due to a biological significance or related to other events requires further investigation. Future work in other Canadian study sites will be necessary to determine whether this same trend is evident in other cities, or whether genotype transmission in other sites is delineated by different types of transmission networks.

5.3 Discussion of Objective 3: Determine whether correlations between social variables and HCV subtypes (i.e. below the genotype level) exist.

The primary aim of the association indices analysis in this study was to provide an exploratory and more detailed analysis of the transmission of genotype 1a and 3a within the IDU population in Winnipeg. Analysis of Association Indices using Simmonics Sequence Editor 2000 [62] is a relatively new innovation, and literature using this technique is limited. The two most significant contributions, Cochrane *et al.* (2002) and van Asten *et al.* (2004) used Association Indices to examine HCV sequence segregation between cities [28, 52]. Both studies found a high degree of HCV exchange between geographically isolated locations.

Increasing AI values indicate a high degree of HCV exchange within the IDU population (i.e. a tendency for an IDU to form one large transmission network). Very small AIs would indicate very distinct, segregated transmission networks within a larger IDU population. Our results showed a modest degree of segregation for the variables "injection at a hotel", "injection in public washroom", "participation in the sextrade" or recently moved to Winnipeg". A modest degree of segregation implies AI values of approximately 0.80 with the percentile range of the test sequences not overlapping that of the control sequences. This non-overlap is considered "significant" although significance in terms of p values is not generated by the AI software. AI values of this type imply segregation defined by specific behavioural characteristic, such that some clustering of transmission is occurring. However, it is apparent from Figure 10 and Figure 11, that this clustering is not strong.

The clustering could represent direct routes of transmission (e.g. between groups of individuals who all inject at hotels and who have shared syringes together), however

because the measured AIs are only modest, there is clearly much viral exchange between groups. A highly mobile IDU whose social network may change over time, coupled with the chronic nature of HCV infections could easily lead to rapidly changing transmission networks with resulting modest AIs. A study of acute HCV infections and current risk behaviours may be more meaningful using this methodology. It would also be useful to carry out a study of this type in other cities, such as Ottawa where geographically distinct IDU scenes are believed to exist and hence relatively clear transmission networks may be identifiable (Ann Jolly, personal communication).

Additionally, the modest AI values reflect the involvement of IDU in multiple roles. For example in Figures 10 and 11, some IDU are involved in the sex trade, have moved to Winnipeg recently, injected at a hotel, and injected in a public washroom, whereas other IDU report only one or none of these behaviours. Thus some clusters of transmission networks appear to exist, but, as seen in other studies, they are not completely isolated from each other due to some mixing between networks.

To our knowledge, this research is the first detailed molecular analysis of HCV below the genotype level and the first to use AI within a single IDU population in a contiguous geographic area. Therefore, the analysis does suggest some limited delineation of the IDU population in Winnipeg. These results would need to be confirmed in future studies, specifically designed to determine how clearly demarcated these transmission networks are.

Chapter 6: Limitations

6.1 Limitations of the overall study

Certain limitations for the overall study affect each of the individual analyses discussed above. Firstly, cross sectional studies do not establish the temporal sequence of events, which makes it difficult to determine the directionality of events (i.e. has a behaviour resulted in infection, or has knowledge of infection status increased the likelihood of engaging in harm reduction activities) [116].

Recall bias may also be a limitation of the questionnaire data. Potential recall bias may occur for behaviour related questions. To attempt to minimize recall bias, questions were asked with regards to behaviours only within the last 6 months.

One research nurse conducted all study participant surveys. As much as possible, participants were interviewed in a location of their choice, which ensured a comfortable secure location for study participants to provide confidential information. Our approach of using word-of mouth advertising could result in our sampling a distinct group of IDU within the greater IDU population in Winnipeg. However, word-of-mouth is considered a good method of finding a representative sample of "hidden populations". It should be noted that there is no mechanism to obtain a true random sample of populations like IDU [117, 118].

Data on sharing of drug preparation equipment (cotton, swabs, cookers and filters) and transfer of prepared drugs between syringes (backloading, frontloading, piggybacking) were only queried for the six months prior to interview. In contrast, sharing of used syringes was based both on six-month and "ever" data. If more extensive

data had been collected for equipment sharing and drug transfer behaviours, they may have also been significant for HCV outcome.

6.2 Social network data

The social network portion of the questionnaire relied on third-party reporting of demographics and behaviour, and the accuracy of these reports cannot be confirmed. However, some questions included behaviours that the individual and the contact had participated in together, which should increase report accuracy.

6.3 Molecular epidemiology of HCV

Our choice to sequence 3 PCR samples from each person may have resulted in missing some genetic diversity. Using PCR-methods, only the prevailing strain will be detected in mixed infections [119]. Mixed infections may have been missed by cloning, but we expect that the predominant genotype within an individual would have been selected. Ultimately the molecular methodology used was able to generate the data with sufficient genetic diversity, and clear non-ambiguous sequence data within the constraints of staff, time and financial support.

Chapter 7: Conclusions and Recommendations

This study had 3 objectives: correlate the seroprevalence of HCV with individual and social network variables (Objective 1), determine which sociodemographic and sociobehavioural variables are correlated with infection by either HCV genotypes 1a or 3a (Objective 2), and determine whether correlations between social variables and HCV subtypes (i.e. below the genotype level) exist (Objective 3). The findings reported in this paper reinforce the clear link between individual behaviours (e.g. injection with someone else's used needle) and HCV transmission; they also suggest the importance of identifying and defining the relatively distinct local transmission networks that exist within a larger IDU population.

In the context of prevention of HCV transmission amongst IDU, harm reduction has had limited impact [13, 36, 91] . A more detailed understanding of the factors associated with transmission may therefore assist in better control of the HCV epidemic [120]. Currently, the WRHA focuses on harm reduction, centered around initiatives such as Street Connections (mobile needle exchange), as well as outreach and public health programs covering testing, harm reduction, and prevention [121]. Response to our questionnaire does indicate that study participants are aware of and use these initiatives and that these endeavours are targeting the IDU community at large. Despite these interventions, it appears that HCV within this population is still relatively high, but lower than in other cities. While the temporal relationship between use of WRHA services and HCV outcome was not established in this study, the results presented in this thesis have assisted in the detailed characterization of HCV transmission amongst IDU in Winnipeg,

and therefore could assist in identifying areas for improvement to programs already in place.

The seroprevalence analysis presented in this thesis confirm the importance of individual risk behaviours, such as injection with syringes previously used by someone else, and the importance of ensuring that clean syringes are as available to users as possible. However, these results also showed the importance of network analysis (e.g. the high risk associated with being connected to any Talwin/Ritalin use). The popularity of Talwin/Ritalin use in Winnipeg and the high seroprevalence of HCV associated with it, highlight the importance of ensuring that the behaviours and demographic characteristics associated with this drug combination are fully understood and should be emphasized in future work.

The logistic regression analysis of HCV genotypes in Winnipeg and the AI analysis revealed some evidence for distinct transmission networks in Winnipeg and what behaviours or demographics delineate those boundaries. Although the boundaries defining these networks are clearly not sharp, it is still important to address and confirm these results in future studies. The segregation of IDU into transmission networks implies there is more *intra*-network connectivity or communication than *inter*-network connectivity. If so, it would be important to ensure that IDU programs are appropriately targeting all at-risk groups. If communication between networks is limited it cannot be assumed that public health messages targeting one group will readily diffuse to other groups.

Overall, the analyses presented in this paper, have all substantially increased our understanding of HCV transmission amongst IDU in this study site. From a

methodological standpoint, this study explores avenues of social networks and transmission using several innovative techniques. This study is the first to examine molecular transmission of HCV within IDU in Winnipeg. It is also amongst the few studies, internationally, to assess risk behaviour correlates with genotype within IDU, and to use Association Indices to examine sequence segregation of HCV amongst IDU. Although both the latter analyses are considered exploratory in nature, the results contribute to the growing literature and methodology for investigating transmission of blood-borne pathogens within IDU. The results as discussed in this paper contribute to the growing literature on HCV, social network analysis, and molecular epidemiology, and will be useful to researchers in this and other jurisdictions where a significant community of IDU exists.

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Appendix 1 - Study Questionnaire

DEMOGRAPHICS:
Read: "The first set of questions is general questions about yourself".

DEM1. What is your date of birth? (yyyy/mm/dd)

__ __ __ __ / __ __ / __ __

DEM2. What gender do you identify yourself as?
(Interviewer: Only ask about gender if necessary to clarify):

0 Male
1 Female
2 Transgender female
3 Transgender male
55 Unsure
66 Not applicable
99 Refused to answer

DEM3. Were you born in Canada?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

DEM4. If yes for DEM3, what was your place of birth (city town or reserve and province)

City, town or reserve:

_____

Province:

_____

55 Unsure
66 Not applicable
99 Refused to answer

DEM5. If No for DEM3, what country were you born in?

_____

55 Unsure
66 Not applicable
99 Refused to answer

DEM6. What is the highest level of education you have completed?

0 Graduated grade 12
1 In grade school now (grade _____)
2 Dropped out before grade 12 (grade_____)
3 Trade school
4 University
5 College
6 Other, (specify_____)
55 Unsure

DEM7. Over the last year what was the main way you got money to live on? ***(circle only one)***

0 Regular work (full, part time or contract)
1 Welfare, EI, pension or other government support
2 Money from family/friends
3 Sex trade/prostitution
4 Dealing or doing drug runs
5 Panhandling
6 Stealing
7 Boosting
8 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

DEM8. Over the last year what other ways did you get money to live on ***(circle all sources)*** ?

0 Regular work (full, part time or contract)
1 Welfare, EI, pension or other government support
2 Money from family/friends
3 Sex trade/prostitution
4 Dealing or doing drug runs
5 Panhandling
6 Stealing
7 Boosting
8 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

DEM9. What ethnic group or family background do you most identify yourself with:

Do not read choices

0 Caucasian/White
1 Chinese
2 Filipino
3 South-Asian (e.g. Indian, Pakistani)
4 other Asian (e.g. Vietnamese, Japanese)
5 Latin American
6 Middle Eastern
7 Black-African
8 Black-Caribbean
9 Other black
10 First Nations (treaty)
11 First Nations (non-treaty)
12 Metis
13 Inuit
14 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

DEM10. What type of residence do you currently live in?

0 At your own house or apartment
1 At family member's house or apartment
2 At a friend's house or apartment
3 Empty House
4 Hostel/Shelter
5 Hotel
6 Shooting gallery
7 Rooming/ boarding house
8 Recovery house/treatment centre
9 On the street
10 Vehicle (trailer, van, car)
11 Detention centre/ Youth camp
12 Jail or prison
13 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

DEM11. Including your current residence, how many different places have you lived in the past year?

_____

DEM12. Using the first 3 digits of your postal code, what part of the city do you live in?
(Interviewer: If participant is concerned about confidentiality, indicate that approximately 15,000-20,000 other people will have this same postal code information)

Option 1 ***(preferred)***
Postal code (first 3 digits)

_____

Option 2
Neighbourhood

_____

55 Unsure
66 Not applicable
99 Refused to answer

DEM13. In what part of the city do you usually hang out?

Option 1 ***(preferred)***
Postal code (first 3 digits)

_____

Option 2
Neighbourhood

_____

55 Unsure
66 Not applicable
99 Refused to answer

DEM14. Have you moved to Winnipeg within the past 12 months?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

If yes, go to DEM15 otherwise go to ED1.

DEM15 Where were you living before you came to Winnipeg

City, town or reserve:

_____

Province:

_____

Country:

_____

55 Unsure
66 Not applicable
99 Refused to answer

INDIVIDUAL DRUG BEHAVIOURS

Read: "Now I would like to ask you about your drug use. All of the answers that you give me are confidential".

ED1. The first time you fixed (injected/shot up), how old were you?

_____

ED2. In the past 6 months, which of the following drugs have you used without injecting? ***(circle all that apply)***

0 Alcohol
1 Acid
2 Painkillers (e.g. dilaudid)
3 Amphetamines
4 Barbiturates
5 Cocaine
6 Crack
7 Demerol/morphine/opium
8 Downers/tranquilizers
9 Ecstasy
10 Gasoline/solvents
11 Marijuana
12 PCP/Angel dust
13 Tylenol 3
14 Heroin
15 Mushrooms
16 Ruffies (Rohypnol)
17 GHB (gamma-hydroxybutyrate)
18 Methadone prescribed
19 Methadone unprescribed
20 None
21 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

ED3. What is your preferred injection drug? (***drug of choice, circle only one)***

0 Cocaine (uptown)
1 Talwin and Ritalin (speedball)
2 Morphine
3 Heroin (horse, junk, smack, downtown)
4 Heroin and cocaine (speedball)
5 Heroin mixed with another drug
6 Amphetamines (speed, uppers)
7 Methadone
8 Crack/rock cocaine
9 Methamphetamine (crystal meth)
10 PCP (angel dust)
11 Dilaudid
12 Barbiturates (downers)
13 Ritalin alone
14 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

ED4. What drug do you most frequently inject? ***(circle only one)***

0 Cocaine (uptown)
1 Talwin and ritalin (speedball)
2 Morphine
3 Heroin (horse, junk, smack, downtown)
4 Heroin and cocaine (speedball)
5 Heroin mixed with another drug
6 Amphetamines (speed, uppers)
7 Methadone
8 Crack/rock cocaine
9 Methamphetamine (crystal meth)
10 PCP (angel dust)
11 Dilaudid
12 Barbiturates (downers)
13 Ritalin alone
14 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

ED5. Which drugs have you injected in the last 6 months? ***(circle all that apply)***

0 Cocaine (uptown)
1 Talwin and Ritalin (speedball)
2 Morphine
3 Heroin (horse, junk, smack, downtown)
4 Heroin and cocaine (speedball)
5 Heroin mixed with another drug
6 Amphetamines (speed, uppers)
7 Methadone
8 Crack/rock cocaine
9 Methamphetamine (crystal meth)
10 CP (angel dust)
11 Dilaudid
12 Barbiturates (downers)
13 Ritalin alone
14 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

ED6. In the past month, how often did you inject (shoot up)?

0 Not at all
1 Once in a while, not every week
2 Regularly, once or twice a week
3 Regularly, three or more times a week
4 Every day
55 Unsure
66 Not applicable
99 Refused to answer

ED7. Over the past 6 months, what types of places have you injected drugs? ***(circle all that apply)***

0 At your own house or apartment
1 At family member's house or apartment
2 At a friend's house or apartment
3 Empty House
4 Hostel/Shelter
5 Hotel
6 Shooting gallery
7 Rooming/ boarding house
8 Recovery house/treatment centre
9 On the street
10 Vehicle (trailer, van, car)
11 Detention centre/ Youth camp
12 Jail or prison
13 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

Individual Drug Behaviours (Continued)

(If hotel was indicated in ED7, ask the following questions (ED8 and ED9), otherwise go to ED10)

ED8. How many different hotels have you injected at in the past 6 months?

_____

ED9. Over the past 6 months, on how many different days did you inject at each of these hotels **(to a maximum of 6 hotels - if more than 6 ask the person to think about the hotels at which they most frequently inject)**

	# of days	Name of hotel (if person is willing to provide hotel name
Hotel A		
Hotel B		
Hotel C		
Hotel D		
Hotel E		
Hotel F		

ED10. Over the past 6 months, what type of place did you inject at most frequently?***(circle only one)***

0 At your own house or apartment
1 At family member's house or apartment
2 At a friend's house or apartment
3 Empty House
4 Hostel/Shelter
5 Hotel
6 Shooting gallery
7 Rooming/ boarding house
8 Recovery house/treatment centre
9 On the street
10 Vehicle (trailer, van, car)
11 Detention centre/ Youth camp
12 Jail or prison
13 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

ED11. This is a "what if" question: If you wanted to always use a clean needle, how certain are you that you could do it ?

0 Extremely sure I could not
1 Quite sure I could not
2 Slightly sure I could not
3 Slightly sure I could
4 Quite sure I could
5 Extremely sure I could

ED12. In the past 6 months, how often have you given away one of your used needles to someone who needed it to inject drugs?

0 Never
1 Occasionally
2 Sometimes
3 Usually
4 Always
55 Unsure
66 Not applicable
99 Refused to answer

If "occasionally" or more, then how many different people have you given one of your needles to in past

_____ week ☐ **OR** month ☐

ED13. In the past 6 months, how often have you used someone else's cooker, rinse water, or cotton that someone else has already used, or may have already used?

0 Never
1 Occasionally
2 Sometimes
3 Usually
4 Always
55 Unsure
66 Not applicable
99 Refused to answer

If "occasionally" or more, then how many different people's cooker, rinse water, or cotton have you used in the past

_____ week ☐ **OR** month ☐

ED14. In the past 6 months, how often did you inject drugs after someone transferred (frontloading, backloading, or piggybacking) drugs into your syringe from their syringe?

0 Never
1 Occasionally
2 Sometimes
3 Usually
4 Always
55 Unsure
66 Not applicable
99 Refused to answer

If "occasionally" or more, then how many different people transferred drugs into your syringe in the past

_____ week ☐ OR month ☐

ED15A. Have you <u>ever</u> used a needle that someone else had or may have already used?

0 No
1 Yes
55 Unsure
99 Refused to answer

If "never", go to ED19

ED15B. In the past 6 months, how often have you used a needle that someone else has already used or may have already used?

0 Never
1 Occasionally
2 Sometimes
3 Usually
4 Always
55 Unsure
66 Not applicable
99 Refused to answer

ED16. In the last **6 MONTHS**, have you used a needle that had been previously used by someone you suspected or knew was HIV (AIDS virus) and/or HCV (hepatitis C) positive?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

If "occasionally" or more, then how many different people's needles, whom you think may have HIV or HCV, have you used in the past

_____ week ☐ OR month ☐

ED17 In the last **6 MONTHS** when you used needles or syringes previously used by someone else, how often did you clean them first?

0 Never
1 Hardly ever
2 Sometimes
3 Frequently
4 Always
55 Unsure
66 Not applicable
99 Refused to answer

If "never", go to ED19.

ED18. How did you usually clean the needles and syringes that someone else had used?

0 Cold water
1 Hot water
2 Boiling water
3 Bleach
4 Alcohol
5 Other, (specify _____)
55 Unsure
66 Not applicable
99 Refused to answer

ED19. Do you think that you can be infected with hepatitis C by injecting drugs after someone has squirted drugs into your syringe from a syringe that they already injected with?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

ED20. Do you think that you can be infected with HIV by injecting drugs after someone has squirted drugs into your syringe from a syringe that they had already injected with?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

ED21. Do you think that you can be infected with hepatitis C by using someone else's cooker, rinse water, or cotton?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

ED22. Do you think that you can be infected with HIV by using someone else's cooker, rinse water, or cotton?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

ED23. In the past 6 months, how many times have you bought drugs for someone else?

0 0 times
1 1 time
2 2-4 times
3 5-9 times
4 10-24 times
5 25-49 times
6 50-99 times
7 100 times or more
55 Unsure
66 Not applicable
99 Refused to answer

ED24. In the past 6 months, how many times have you sold drugs?

0 0 times
1 1 time
2 2-4 times
3 5-9 times
4 10-24 times
5 25-49 times
6 50-99 times
7 100 times or more
55 Unsure
66 Not applicable
99 Refused to answer

ED25. In the past 6 months, how many times have you bought needles for someone else?

0 0 times
1 1 time
2 2-4 times
3 5-9 times
4 10-24 times
5 25-49 times
6 50-99 times
7 100 times or more
55 Unsure
66 Not applicable
99 Refused to answer

ED26. In the past 6 months, how many times have you given away needles?

0 0 time
1 1 time
2 2-4 times
3 5-9 times
4 10-24 times
5 25-49 times
6 50-99 times
7 100 times or more
55 Unsure
66 Not applicable
99 Refused to answer

ED27. In the past 6 months, how many times have you injected someone else with drugs as a service in return for drugs, money or other goods?

0 0 time
1 1 time
2 2-4 times
3 5-9 times
4 10-24 times
5 25-49 times
6 50-99 times
7 100 times or more
55 Unsure
66 Not applicable
99 Refused to answer

ED28. In the past 6 months, how many time have you injected someone else with drugs as a favour to help them out (i.e. you weren't expecting anything in return)?

0 0 time
1 1 time
2 2-4 times
3 5-9 times
4 10-24 times
5 25-49 times
6 50-99 times
7 100 times or more
55 Unsure
66 Not applicable
99 Refused to answer

NEEDLE SOURCES

NS1. In the last 6 months, have you exchanged needles or gotten new needles at a needle exchange?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

NS2. In the last 6 months, how many of your needles did you usually get at a needle exchange?

0 All
1 Most, but not all
2 About half
3 Less than half
4 None
55 Unsure
66 Not applicable
99 Refused to answer

NS3. In the last 6 months, have you usually exchanged your own needles, or does someone else do it for you?

0 Usually do it myself
1 Usually done for me by someone else
55 Unsure
66 Not applicable
99 Refused to answer

NS4. In the last 6 months, where did you get your new needles from.? (***Circle all that apply)***

0 Nurse/doctor/hospital
1 Pharmacy/drugstore
2 Street Connections
3 Other needle exchanges, (specify_____)
4 Someone on the street
5 Dealer
6 Shooting Gallery Owner
7 Friends/partners/family
8 Found on the street
55 Unsure
66 Not Applicable (don't ever use new syringes)
99 Refused to answer

NS5. Of all the places you obtained new needles from in the last 6 months, from where did you get most of your needles? ***(Circle only one)***

0 Nurse/doctor/hospital
1 Pharmacy/drugstore
2 Street Connections
3 Other needle exchanges, (specify_____)
4 Someone on the street
5 Dealer
6 Shooting Gallery Owner
7 Friends/partners/family
8 Found on the street
55 Unsure
66 Not Applicable (don't ever use new syringes
99 Refused to answer

NS6. In the last 6 months how easy was it for you to obtain a brand new needle/syringe when you needed one?

0 Very easy
1 Somewhat easy
2 Somewhat difficult
3 Very difficult
55 Unsure
66 Not applicable
99 Refused to answer

For the following sections, check the appropriate boxes unless full answer is requested

CONTACT DEMOGRAPHICS

CD1. What is [person]'s relationship to you?
This is partially a repeat from the initial network member list, but more detailed types of relationships are listed here, so the participant must be asked the question again for the 5 chosen network members.

	Network Member #				
	1	2	3	4	5
0 Friend					
1 2 Spouse					
2 Girl/Boyfriend, lover					
3 Ex-lover					
4 Ex-spouse					
5 Mother					
6Father					
7 Brother					
8 Sister					
9 Son					
10 Daughter					
11 Cousin					
12 In-laws					
13 Niece					
14 Nephew					
15 Uncle					
16 Aunt					
17 Other relative					
18 Acquaintance					
19 Stranger					
20 Dealer					
21 Trick					
22 Other, specify below					
55 Unsure					
99 Refused to answer					

If "other" is selected above, specify what "other" means in the box below.

Network Member #	Other, specify
1	
2	
3	
4	
5	

CD2. How long have you known [person]?

Network Member #	
1	
2	
3	
4	
5	

CD3. Where was [person] born?

Network Member #	
1	
2	
3	
4	
5	

CD4. How frequently would you say you have contact with [person]?

	Network Member #				
	1	2	3	4	5
0 Daily					
1. 2-4 times per week					
2. Once a week					
3. 1-3 times per month					
4. Less than once per month					
55 Unsure					
66 Not applicable					
99 Refused to answer					

CD5. How did you meet [person]?

	Network Member #				
	1	2	3	4	5
0 Work together					
1 School					
2 Member of same gang					
3 On the street					
4 Neighbours					
5 Through mutual friends					
6 Through a family member					
7 Injected together					
8 Person is a family member					
9 Bar/Hotel					
10 Other, specify below					
55 Unsure					
66 Not applicable					
99 Refused to answer					

If "other" is selected above, specify what "other" means in the box below.

Network Member #	Other, specify
1	
2	
3	
4	
5	

CD6. What is the highest level of education [person] has completed?

	Network Member #				
	1	2	3	4	5
0 Graduated grade 12					
1 In grade school now (Grade _____)					
2 Dropped out before grade 12 (grade _____)					
3 Trade school					
4 University					
5 College					
6 Other, specify below					
55 Unsure					
66 Not applicable					
99 Refused to answer					

If "other" is selected above, specify what "other" means in the box below.

Network Member #	Other, specify
1	
2	
3	
4	
5	

CD7. Over the last year what was [person's] main source of income?

	Network Member #				
	1	2	3	4	5
0 Regular work (full, part time or contract)					
1 Welfare, EI, pension or other government support					
2 Money from family/friends					
3 Sex trade/prostitution					
4 Dealing or doing drug runs					
5 Panhandling					
6 Stealing					
7 Boosting					
8 Other					
55 Unsure					
66 Not applicable					
99 Refused to answer					

CD8. What part of the city do they **live in**? Use the first 3 digits of their postal code (preferred) or neighbourhood name, if you know it. (**If they live outside of city, ask for name of town or reserve**).

Network Member #	Postal Code (1st three digits only) or Neighbourhood name	Unsure	Not app.	Refused to answer
1				
2				
3				
4				
5				

CD9. What part of the city do they **hang out** in? Use the first 3 digits of their postal code (preferred) or neighbourhood name, if you know it. (**If they live outside of city, ask for name of town or reserve**).

Network Member #	Postal Code (1st three digits only) or Neighbourhood name	Unsure	Not app.	Refused to answer
1				
2				
3				
4				
5				

CONTACT INJECTION DRUG RISK

CDR1. To the best of your knowledge, in the past month, how often did [person] shoot up?

	Network Member #				
	1	2	3	4	5
0 Not at all					
1 Once in a while, not every week					
2 Regularly, once or twice a week					
3 Regularly, three or more times per week					
4 Every day					
55 Unsure					
66 Not Applicable					
99 Refused to answer					

CDR2. To the best of your knowledge, what drug does [person] most frequently inject? ***(Check only one)***

	Network Member #				
	1	2	3	4	5
0 Cocaine (uptown)					
1 Talwin and ritalin (speedball)					
2 Morphine					
3 Heroin (horse, junk, smack, downtown)					
4 Heroin and cocaine (speedball)					
5 Heroin mixed with another drug					
6 Amphetamines (speed, uppers)					
7 Methadone					
8 Crack/rock cocaine					
9 Methamphetamine (crystal meth)					
10 PCP (angel dust)					
11 Dilaudid					
12 Barbiturates (downers)					
13 Ritalin alone					
14 Other, specify below					
55 Unsure					
66 Not applicable					
99 Refused to answer					

If "other" is selected above, specify what "other" means in the box below.

Network Member #	Other, specify
1	
2	
3	
4	
5	

CDR3. To the best of your knowledge, what is [person's] preferred injection drug?

	Network Member #				
	1	2	3	4	5
0 Cocaine (uptown)					
1 Talwin and Ritalin (speedball)					
2 Morphine					
3 Heroin (horse, junk, smack, downtown)					
4 Heroin and cocaine (speedball)					
5 Heroin mixed with another drug					
6 Amphetamines (speed, uppers)					
7 Methadone					
8 Crack/rock cocaine					
9 Methamphetamine (crystal meth)					
10 PCP (angel dust)					
11 Dilaudid					
12 Barbiturates (downers)					
13 Ritalin alone					
14 Other, specify below					
55 Unsure					
66 Not applicable					
99 Refused to answer					

If "other" is selected above, specify what "other" means in the box below.

Network Member #	Other, specify
1	
2	
3	
4	
5	

CDR4. Approximately, how long have they been injecting drugs? (Record as day, month or year and specify which {d, m or y})

Network Member #	Day, month or year
1	
2	
3	
4	

CDR5. To the best of your knowledge, in the past 6 months, has [person] injected: ***(check all that apply)***

	Network Member #				
	1	2	3	4	5
0 In a private residence (such as your own, their own or a friend's house or apartment)					
1 At a hotel					
2 At a shooting gallery					
3 At another public place such as on the street, a public washroom, empty house, or hostel					
55 Unsure					
66 Not applicable					
99 Refused to answer					

CDR6. In the past 6 months, how many times have you and [person] combined or pooled money so that you had enough money to buy drugs or injecting equipment?

	Network Member #				
	1	2	3	4	5
0 0 times, never					
1. 1 time					
2. 2-4 times					
3. 5-9 times					
4. 10-24 times					
5. 25-49 times					
6. 50-99 times					
7. 100 times or more					
55 Unsure					
66 Not applicable					
99 Refused to answer					

CDR7. In the past 6 months, how often did you inject with [person]?

	Network Member #				
	1	2	3	4	5
0 Not at all					
1 Once in a while, not every week					
2 Regularly, Once or twice a week					
3 Regularly, Three or more times per week					
4 Every day					
55 Unsure					
66 Not Applicable					
99 Refused to answer					

CDR8. Have you ever injected with a needle after [person] used it first?

	Network Member #				
	1	2	3	4	5
0 No					
1 Yes					
55 Unsure					
66 Not applicable					
99 Refused to answer					

If "No", go to CDR11.

CDR9. In the past 6 months, how often have you injected with a needle after [person] used it first?

	Network Member #				
	1	2	3	4	5
0 Never					
1 Occasionally					
2 Sometimes					
3 Usually					
4 Always					
55 Unsure					
66 Not Applicable					
99 Refused to answer					

If CDR9 is other than never, go to CDR10, otherwise go to CDR11.

CDR10. The last time this happened, can you describe in your own words why you ended up using their used needle? Why did they use it first?

(Please respond on separate answer sheet)

CDR11. In the past 6 months, how often has [person] injected with a needle after you used it first?

	Network Member #				
	1	2	3	4	5
0 Never					
1 Occasionally					
2 Sometimes					
3 Usually					
4 Always					
55 Unsure					
66 Not Applicable					
99 Refused to answer					

If CDR11 is other than never, go to CDR12, otherwise go to CDR13.

CDR12. The last time this happened, can you describe in your own words why they ended up using your used needle? Why did you use it first?

(Please respond on separate answer sheet)

CDR13. In the past 6 months, how often have you used [person's]cooker, rinse water, or cotton after they had already used them?

	Network Member #				
	1	2	3	4	5
0 Never					
1 Occasionally					
2 Sometimes					
3 Usually					
4 Always					
55 Unsure					
66 Not Applicable					
Refused to answer					

CDR14. In the past 6 months, how often did you inject drugs after [person] mixed your drugs in a syringe that they had already injected with?

	Network Member #				
	1	2	3	4	5
0 Never					
1 Occasionally					
2 Sometimes					
3 Usually					
4 Always					
55 Unsure					
66 Not Applicable					
Refused to answer					

CDR15. How long have you been injecting drugs with [person]? (Record as day, month or year and specify which {d, m or y})

Network Member #	
1	
2	
3	
4	
5	

CDR16. Where do you and [person] inject together? ***(Check all that apply)***

	Network Mmber #				
	1	2	3	4	5
0 In a private residence (such as your own, their own or a friend's house or apartment)					
2 At a hotel					
3 At a shooting gallery					
4 At another public place such as on the street, a public washroom, empty house, or hostel					
55 Unsure					
66 Not applicable					
Refused to answer					

CONTACT DRUG SMOKING

CDSS1. Not including marijuana use, in the past month, how often did [person] smoke, snort or inhale drugs?

	Network Member #				
	1	2	3	4	5
0 Not at all					
1 Once in a while, not every week					
2 Regularly, once or twice a week					
3 Regularly, Three or more times per week					
4 Every day					
55 Unsure					
66 Not Applicable					
99 Refused to answer					

If "Not at all", go to INT1

CDSS2. Not including marijuana, what drug does person] most frequently smoke, snort or inhale?

	Network Member #				
	1	2	3	4	5
0 Acid					
1 Painkillers (e.g.dilaudid)					
2 Demerol/morphine/opium					
3 Downers/tranquilizers					
4 Ecstasy					
5 Gasoline/solvents					
6 Tylenol 3					
7 Ruffies (Rohypnol)					
8 GHB (gamma-hydroxybutyrate					
9 Methadone (prescribed)					
10 Methadone (unprescribed)					
11 Cocaine (uptown)					
12 Talwin and ritalin (speedball)					
13 Morphine					
14 Heroin (horse, junk, smack, downtown)					
15 Heroin and cocaine (speedball)					
16 Heroin mixed with another drug					
17 Amphetamines (speed, uppers)					
18 Methadone					
19 Crack/rock cocaine					
20 Methamphetamine (crystal meth)					
21 PCP (angel dust)					
22 Dilaudid					
23 Barbiturates (downers)					
24 Ritalin alone					
25 No other drug					
26 Other, specify below					
55 Unsure					
66 Not applicable					
99 Refused to answer					

CDSS3. Not including marijuana use, how long has [person] been smoking, snorting or inhaling drugs? (Record as days, months or years and specify with d, m, or y)

Network Member #	Day, month or year
1	
2	
3	
4	
5	

INTIMACY

INT1. How close are you to [person]?

	Network Member #				
	1	2	3	4	5
0 Very distant					
1 Distant					
2 Somewhat close					
3 Close					
4 Very close					
55 Unsure					
66 Not applicable					
Refused toanswer					

INT2. Would you talk to [person] about things that are very personal and private?

	Network Member #				
	1	2	3	4	5
0 No					
1 Yes					
55 Unsure					
66 Not applicable					
Refused to answer					

INT3. If you needed to borrow $25, would [person] lend or give it to you if they had the money?

	Network Member #				
	1	2	3	4	5
0 No					
1 Yes					
55 Unsure					
66 Not applicable					
Refused to answer					

INT4. Would you ask [person] for advice or help about health problems like infections, AIDS, or hepatitis C?

	Network Member #				
	1	2	3	4	5
0 No					
1 Yes					
55 Unsure					
66 Not applicable					
Refused to answer					

RELATIONSHIP CHARACTERISTICS AND BELIEFS

Read: Can you tell me whether you strongly agree, agree, disagree, or strongly disagree with the following statements (yes/no are used for some statements)?

RE1. Sharing needles is an important part of the relationship that I have with [person].

	Network Member #				
	1	2	3	4	5
Strongly agree					
Agree					
Disagree					
Strongly disagree					

RE2. If [person] stopped injecting drugs, I would probably also stop.

	Network Member #				
	1	2	3	4	5
Strongly agree					
Agree					
Disagree					
Strongly disagree					

RE3. If [person] stopped injecting drugs, it would be more difficult to maintain a relationship with them.

	Network Member #				
	1	2	3	4	5
Strongly agree					
Agree					
Disagree					
Strongly disagree					

RE4. Injecting drugs is an important part of the relationship I have with [person].

	Network Member #				
	1	2	3	4	5
Strongly agree					
Agree					
Disagree					
Strongly disagree					

RE5. [Person] would object if I wanted to stop injecting drugs.

	Network Member #				
	1	2	3	4	5
Strongly agree					
Agree					
Disagree					
Strongly disagree					

RE6. [Person] obtains drugs for me.

	Network Member #				
	1	2	3	4	5
No					
Yes					
Unsure					
Refused to answer					

RE7. I obtain drugs for [person].

	Network Member #				
	1	2	3	4	5
No					
Yes					
Unsure					
Refused to answer					

RE8. [Person] obtains needles or other equipment for.

	Network Member #				
	1	2	3	4	5
No					
Yes					
Unsure					
Refused to answer					

RE9. I obtain needles or other equipment for [person].

	Network Member #				
	1	2	3	4	5
No					
Yes					
Unsure					
Refused to answer					

RE10. I believe that [Person] is infected with HIV (AIDS virus).

	Network Member #				
	1	2	3	4	5
No					
Yes					
Unsure					
Refused to answer					

RE11. I believe that [Person] is infected with hepatitis C.

	Network Member #				
	1	2	3	4	5
No					
Yes					
Unsure					
Refused to answer					

NORMS AND EXPECTATIONS OF CONTACTS FOR NEEDLE USE.

Read: Do you strongly agree, agree, disagree or strongly disagree with the following statements.

NE1. [Person] talks about harm reduction and safe injection.

	Network member #				
	1	2	3	4	5
0 Strongly agree					
1 Agree					
2 Disagree					
3 Strongly disagree					

NE2. [Person] encourages me to always use clean needles.

	Network member #				
	1	2	3	4	5
0 Strongly agree					
1 Agree					
2 Disagree					
3 Strongly disagree					

NE3. [Person] would encourage me to always use my own equipment like cotton, rinse water or cookers?

	Network member #				
	1	2	3	4	5
0 Strongly agree					
1 Agree					
2 Disagree					
3 Strongly disagree					

NE4. In the past 6 months, how many times would you estimate [Person] has used someone else's needle?

	Network Member #				
	1	2	3	4	5
1 Never					
2 Occasionally					
3 Sometimes					
4 Usually					
5 Always					
55 Unsure					
66 Not Applicable					
Refused to answer					

NE5. [Person] would never use a used needle that they found on the street or in a shooting gallery or in some other public place?

	Network Member #				
	1	2	3	4	5
0 Strongly agree					
1 Agree					
2 Disagree					
3 Strongly disagree					

NE6. [Person] would not give a used needle to another IDU?

	Network Member #				
	1	2	3	4	5
0 Strongly agree					
1 Agree					
2 Disagree					

INITIATION AND DEMONSTRATION OF INJECTION

ID1. [Person] introduced me or initiated me into injection drug use.

	Network Member #				
	1	2	3	4	5
0 No					
1 Yes					
55 Unsure					
66 Not applicable					
Refused to answer					

ID2. [Person] has shown me how to inject drugs.

	Network Member #				
	1	2	3	4	5
0 No					
1 Yes					
55 Unsure					
66 Not applicable					
Refused to answer					

ID3. [Person] has injected me with drugs.

	Network Member #				
	1	2	3	4	5
0 No					
1 Yes					
55 Unsure					
66 Not applicable					
Refused to answer					

If No, stop administering the questionnaire, otherwise go to ID4.

END OF QUESTIONNAIRE
Thank you.

Interviewer: Ask participant if they have any questions. Provide any informational pamphlets, information about local agencies, and referrals as appropriate

ID4. In the past 6 months, how many times has [person] injected you with drugs?

	Network Member #				
	1	2	3	4	5
0 Never					
1 Occasionally					
2 Sometimes					
3 Usually					
4 Always					
55 Unsure					
66 Not Applicable					
Refused to answer					

If "never", stop administering the questionnaire, otherwise go to ID5

END OF QUESTIONNAIRE
Thank you.

Interviewer: Ask participant if they have any questions. Provide any informational pamphlets, information about local agencies, and referrals as appropriate.

ID5. In the past 6 months, when [person] injected you with drugs, how many times was it done as a favour to help you out (i.e. they weren't expecting anything in return)?

	Network Member #				
	1	2	3	4	5
0 Never					
1 Occasionally					
2 Sometimes					
3 Usually					
4 Always					
55 Unsure					
66 Not Applicable					
Refused to answer					

ID6. In the past 6 months, how many times has [person] injected you with drugs in exchange for money, drugs, or other goods?

	Network Member #				
	1	2	3	4	5
0 Never					
1 Occasionally					
2 Sometimes					
3 Usually					
4 Always					
55 Unsure					
66 Not Applicable					
Refused to answer					

ID7. Is there anything else you would like to add regarding the reasons why you have [person] inject you with drugs?

(Please respond on separate answer sheet)

ID8. In the past 6 months when they injected you with drugs, on how many of those occasions had they used the needles to inject themselves first?

	Network Member #				
	1	2	3	4	5
0 Never					
1 Occasionally					
2 Sometimes					
3 Usually					
4 Always					
55 Unsure					
66 Not Applicable					
Refused to answer					

If ID8 is other than "never", go to ID9, otherwise stop administering the questionnaire.

END OF QUESTIONNAIRE
Thank you.

Interviewer: Ask participant if they have any questions. Provide any informational pamphlets, names of local agencies, and referrals as appropriate.

ID9. The last time this happened, can you describe for me why [person] used the needle first?

(Please respond on separate answer sheet)

END OF QUESTIONNAIRE
Thank you.

Interviewer: Ask participant if they have any questions. Provide any informational pamphlets, names of local agencies, and referrals as appropriate.

Appendix 2 - Consent form

Manitoba

Health

Cadham Provincial Public Health Laboratory
Public Health Branch

P.O. Box 8450
750 William Avenue
Winnipeg MB R3C 3Y1
PH: (204) 945-6123
FAX: (204) 786-4770

Networks and infectious disease: social and molecular factors affecting transmission of hepatitis C and HIV among injection drug users.

Information and consent form

Principal investigator: Dr. John Wylie, Manitoba Health, 945-7473.

Purpose of the study: We are currently conducting a study on intravenous drug use in Winnipeg, and would appreciate your help. This study is designed to:

•. Give us more information on some of the things we found in our first study; such as why and with whom people share drug equipment.
• Help you determine whether you are infected with hepatitis C, HIV, or hepatitis B.
• Find out how many intravenous drug users in the city have Hepatitis C.
• Viruses, like human beings, are each slightly different from each other. We wish to identify the number of different types of hepatitis C that are present within the drug user population
• Identify how well the immune systems of drug users are able to resist infection.

Study procedures: We would first like to ask you a series of questions. These questions will be about yourself, your injection drug use, and the people you normally hang out with. We will not ask you for the last names of the people you hang out with. We will not be collecting any information that could be used to identify or find these people. You may refuse to answer any questions at any time. The questionnaire should take approximately 2 hours to complete.

We would also like you to provide a blood specimen. We will use this specimen for several different tests. You can choose to have all of these tests done, or just some of the tests, or, you can refuse all of the tests. If you choose to give a specimen, we can:

• give you your hepatitis C, HIV, hepatitis B infection status. The specimen sent in for testing will only have a number attached to it, not your name, so if you wish to have treatment or see a doctor about the infections we identify, you would have to see a doctor to be tested again. The study nurse would be able to help you arrange for this testing.
• determine which types of hepatitis C are present in Winnipeg.
• find out how well the immune systems of IDU are able to stop infection.

If you want to know whether you have hepatitis C, HIV, or hepatitis B, you can set another appointment with the study nurse and at that time she will give give you your results. She will also talk with you about your health concerns during your time with her, and, if you wish, will put you in touch with other services if you need or want them.

Benefits of the study: This information will help us to better understand how big a problem hepatitis C is in Winnipeg and, by comparing our results to the WIDE study, we will be able to

tell whether HIV is continuing to spread among IDU. This information and the information on why and how people share drug equipment will help to provide better health, and other, services to injection drug users. Hepatitis B testing will help us determine whether people are having themselves vaccinated against Hepatitis B and how many people have been infected by this virus.

Costs: Taking part in this study will not cost you anything.

Payment for participation: You will be given $40 for participating in the study.

Study risks: The only physical risks associated with taking part in this study, is the slight discomfort from having a blood specimen drawn, and the very small chance of an infection from having the skin pierced to take blood.

Confidentiality: All of the information you give us for the study will remain confidential. No information which can identify you or anyone else will be used in this study and your name will not be written on the questionnaire. All of the information that you give us will be put into the study using a number such as #23. If you wish to have your blood test results the study nurse will ask you for a first name, street name, or a made-up name that you will use to identify yourself when you come back for your second appointment. The study nurse will keep a list of these names and code numbers so that she can be sure she is giving you the correct test results. The blood specimens that are sent for testing will only have the study code written on them. This list kept by the nurse will be destroyed as soon as the study is completed. This list will be kept separately from the questionnaire data. Because we have only a number for you and all responses will be grouped for analysis, no one will ever know how, you, as an individual, answered the questions.

The nurse will also ask your help in bringing other people into the study. She will either give you a card with your study number written on the back (for example, #23) which you can give to a friend, or she will ask you to bring in another friend to the study. We are interested in doing this as we would like to see how injection drug users are connected together. All of this will be done with numbers, so we will link you with your friend only by study numbers. You do not have to take part in this part of the study if you do not want to.

We will do everything possible to ensure your confidentiality and the records for this study will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. The University of Manitoba Health Research Ethics Board may review records related to the study to ensure that the study is being conducted in an ethical manner.

Voluntary participation: Whether you decide to take part in the study is entirely up to you. You may drop out at any time and whether you decide to take part or not, will not affect whether you can get health care from anyone.

Questions: If you have any questions about the study itself or why we are doing it, please talk with the study nurse, or contact the head of the study group, Dr. John Wylie, Cadham Provincial Laboratory, (945-7473). For questions about your rights as a research participant, you may contact the University of Manitoba Health Research Ethics Board at 789-3389. Only complete this consent form if you have had a chance to ask questions and have received satisfactory answers to all of your questions.

INFORMED CONSENT

I have read or heard the reasons for this study and how it works. I have been given the chance to talk about it and have my questions answered to my satisfaction. I choose to take part in the parts of the study shown below. I will be given a copy of this consent form if I want one. If necessary, I authorize the inspection of the information I have provided by the University of Manitoba Health Research Ethics Board to ensure that the study is being conducted in an ethical manner. I understand that by consenting to take part in this study I have not waived any of the legal rights that I have as a participant in a research study.

Consent for questionnaire	Yes ( )	No ( )
Consent for providing a blood specimen	Yes ( )	No ( )
Consent for receiving HIV test results	Yes ( )	No ( )
Consent for receiving HCV test results	Yes ( )	No ( )
Consent for receiving HBV test results	Yes ( )	No ( )
Consent for typing of hepatitis C in blood specimen	Yes ( )	No ( )
Consent for resistance to infection	Yes ( )	No ( )

_____ _____ _____

Name (please print) Signature Date

Note: If you want to take part in this study but do not want to sign your name, please tell the research nurse. If you want, you can just tell her that you want to take part and she can write that you said so.

Oral consent provided: Yes ( ) No ( )

Copy of consent form offered to client: Yes (_____)

Subject's name: _____

Study nurse: _____

Signature of study nurse: _____

Time and date of questionnaire (e.g. 13:45, dd/mm/yyyy): _____

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• Find out how many intravenous drug users in the city have Hepatitis C.
• Viruses, like human beings, are each slightly different from each other. We wish to identify the number of different types of hepatitis C that are present within the drug user population
• Identify how well the immune systems of drug users are able to resist infection.

Study procedures: We would first like to ask you a series of questions. These questions will be about yourself, your injection drug use, and the people you normally hang out with. We will not ask you for the last names of the people you hang out with. We will not be collecting any information that could be used to identify or find these people. You may refuse to answer any questions at any time. The questionnaire should take approximately 2 hours to complete.

We would also like you to provide a blood specimen. We will use this specimen for several different tests. You can choose to have all of these tests done, or just some of the tests, or, you can refuse all of the tests. If you choose to give a specimen, we can:

• give you your hepatitis C, HIV, hepatitis B infection status. The specimen sent in for testing will only have a number attached to it, not your name, so if you wish to have treatment or see a doctor about the infections we identify, you would have to see a doctor to be tested again. The study nurse would be able to help you arrange for this testing.
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Consent for providing a blood specimen	Yes ( )	No ( )
Consent for receiving HIV test results	Yes ( )	No ( )
Consent for receiving HCV test results	Yes ( )	No ( )
Consent for receiving HBV test results	Yes ( )	No ( )
Consent for typing of hepatitis C in blood specimen	Yes ( )	No ( )
Consent for resistance to infection	Yes ( )	No ( )

_____ _____ _____

Name (please print) Signature Date

Note: If you want to take part in this study but do not want to sign your name, please tell the research nurse. If you want, you can just tell her that you want to take part and she can write that you said so.

Oral consent provided: Yes ( ) No ( )

Copy of consent form offered to client: Yes (_____)

Subject's name: _____

Study nurse: _____

Signature of study nurse: _____

Time and date of questionnaire (e.g. 13:45, dd/mm/yyyy): _____

BINGES

Read: Binges are "when you fixed more often than your usual drug use for a short period of time and then you went cold or back to usual use"

BN1 Over the last **6 months**, did you go on runs or binges of injection drugs?
If No go to SIS1, otherwise BN2

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

BN2 How often did you binge over the last **6 months**?

0 At least once a week
1 Every couple of weeks
2 About once a month (about 6 times)
3 Once every 2-3 months (about 2-3 times)
4 Once every 3-6 months (about 1-2 times)
5 Less than once every 6 months
6 Never
55 Unsure
66 Not applicable
99 Refused to answer

BN3 In the last **6 months**, on average, how long were your binges/drug runs (of injection drugs)?

# of days: _____

# of injections per day:

_____

Total/binge (#days x # injections per day):

_____

What injection drug(s) do you normally binge on? ***(list in order of most frequent)***

1_____

_____

2_____

_____

3_____

_____

SMOKING, INHALING OR SNORTING DRUGS

SIS1. Have you smoked, inhaled or snorted any drugs in the last 6 months?
If No, go to SEB1 (page 10), otherwise SIS2

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SIS2. What drugs have you smoked/inhaled/snorted? ***(circle all that apply)***

0 Acid
1 Barbiturates
2 Cocaine (uptown)
3 Crack/rock cocaine
4 Demerol/morphine/opium
5 Downers/tranquilizers
6 Ecstasy
7 Gasoline/solvents
8 Marijuana
9 PCP/Angel dust
10 Tylenol 3
11 Ruffies (Rohypnol)
12 GHB (gamma-hydroxybutyrate)
13 Methadone prescribed
14 Methadone unprescribed
15 Talwin and Ritalin (speedball)
16 Morphine
17 Heroin (horse, junk, smack, downtown)
18 Heroin and cocaine (speedball)
19 Heroin mixed with another drug
20 Amphetamines (speed, uppers)
21 Methamphetamine (crystal meth)
22 Dilaudid
23 Ritalin alone
24 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

If only marijuana was indicated in SIS2, go to SEB1 (page 10).

SIS3. Not including marijuana, what drug do you most frequently smoke/inhale/snort? ***(Circle only one)***

0 Acid
1 Painkillers (e.g. dilaudid)
2 Barbiturates
3 Cocaine (uptown)
4 Crack/rock cocaine
5 Demerol/morphine/opium
6 Downers/tranquilizers
7 Ecstasy
8 Gasoline/solvents
9 Marijuana
10 PCP/Angel dust
11 Tylenol 3
12 Ruffies (Rohypnol)
13 GHB (gamma-hydroxybutyrate)
14 Methadone prescribed
15 Methadone unprescribed
16 Talwin and Ritalin (speedball)
17 Morphine
18 Heroin (horse, junk, smack, downtown)
19 Heroin and cocaine (speedball)
20 Heroin mixed with another drug
21 Amphetamines (speed, uppers)
22 Methamphetamine (crystal meth)
23 Dilaudid
24 Ritalin alone
25 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

SIS4 Excluding marijuana use, in the past month, how often did you smoke/inhale/snort drugs?

0 Not at all
1 Once in a while, not every week
2 Regularly, once or twice a week
3 Regularly, three or more times a week
4 Every day
55 Unsure
66 Not applicable
99 Refused to answer

SIS5. Excluding marijuana, over the past 6 months at what types of places have you smoked/inhaled/snorted drugs?
(circle all that apply)

0 At your own house or apartment
1 At family member's house or apartment
2 At a friend's house or apartment
3 Empty House
4 Hostel/Shelter
5 Hotel
6 Shooting gallery
7 Rooming/ boarding house
8 Recovery house/treatment centre
9 On the street
10 Vehicle (trailer, van, car)
11 Detention centre/ Youth camp
12 Jail or prison
13 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

SIS6. Excluding marijuana use, over the past 6 months, at what types of places did you most frequently smoke/snort/inhale drugs? **(Circle one only)**

0 At your own house or apartment
1 At family member's house or apartment
2 At a friend's house or apartment
3 Empty House
4 Hostel/Shelter
5 Hotel
6 Shooting gallery
7 Rooming/ boarding house
8 Recovery house/treatment centre
9 On the street
10 Vehicle (trailer, van, car)
11 Detention centre/ Youth camp
12 Jail or prison
13 Other, (specify_____)
55 Unsure
66 Not applicable
99 Refused to answer

SIS7. Not including marijuana use, in the last 6 months, have you used a straw, pipe or can that someone else had already used to smoke, snort or inhale drugs?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

If yes, go to SIS8, otherwise SIS9.

SIS8. Excluding marijuana use, how often, in the last 6 months, did you use a straw, pipe or can that someone else had already used to smoke, snort or inhale drugs?

0 Hardly ever
1 Sometimes
2 Frequently
3 Always
55 Unsure
99 Refused to answer

SIS9. In the last 6 months, have you ever had cuts or burns on your lips or inside your mouth due to crack smoking?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SEXUAL BEHAVIOURS

SEB1. Over the last 6 months, how many sexual partners of the opposite sex have you had (includes vaginal, anal and/or oral sex with regular, casual, and client/"date"/trick partners)?
If 0, then go to SEB4, otherwise SEB2

_____

55 Unsure
66 Not applicable
99 Refused to answer

SEB2. How many of these opposite sex partners were:

a) Regular partners (someone with whom you have a relationship and with whom you are emotionally involved)?

_____

55 Unsure
66 Not applicable
99 Refused to answer

b) Casual sex partners (someone you have had sex with once or a few times, but with whom you have no emotional involvement)?

_____

55 Unsure
66 Not applicable
99 Refused to answer

c) Client sex partners (a client is someone who has given you money, drugs, goods or anything else in exchange for sex)?

_____

55 Unsure
66 Not applicable
99 Refused to answer

SEB3. In the past 6 months how often did you use a condom or barrier with opposite sex partners? ***(check appropriate box)***

	Partner		
	Regular	**Casual**	**Client**
0 Never			
1 Occasionally			
2 Sometimes			
3 Usually			
4 Always			
55 Unsure			
66 Not Applicable			
Refused to answer			

SEB4. Over the last 6 months, how many sexual partners of the same sex have you had (includes vaginal, anal and/or oral sex with regular, casual, and client/"date"/trick partners)?
If 0, then go to SS1, otherwise SEB5

_____

55 Unsure
66 Not applicable
99 Refused to answer

SEB5. Were any of these same sex partners?

a) Regular partners (someone with whom you have a relationship and with whom you are emotionally involved)?

_____

55 Unsure
66 Not applicable
99 Refused to answer

b.) Casual sex partners (someone you have had sex with once or a few times, but with whom you have no emotional involvement)?

_____

55 Unsure
66 Not applicable
99 Refused to answer

c.) Client sex partners (a client is someone who has given you money, drugs, goods or anything else in exchange for sex)?

_____

55 Unsure
66 Not applicable
99 Refused to answer

SEB6. In the past 6 months how often did you use a condom or barrier with same sex partners? ***(Check appropriate box)***

	Partner		
	Regular	**Casual**	**Client**
0 Never			
1 Occasionally			
2 Sometimes			
3 Usually			
4 Always			
55 Unsure			
66 Not Applicable			
Refused to answer			

HEALTH AND SUPPORT

Social support

SS1. Are there people who would loan you $50 if you needed it?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SS2. Are there people who you could talk to, to get information about infections like HCV (hepatitis C) and HIV (AIDS virus)?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SS3. Are there people you can depend on in an emergency, even if they had to go out of their way?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SS4. Are there people you could talk to about things that have been troubling you, or people you could confide in?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SS5. Are there people who really understand you, who understand your feelings and what your life is like?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SS6. If you were feeling down on yourself, or felt that you couldn't do anything right, are there people who would have faith in you?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SS7. Are there people who accept you as you are, both your good and bad points?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SS8. Are there people who let you know they respect who you are, and how you think and act?

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

HEALTH AND SUPPORT
Social diversity

Read: In the past two weeks have you spoken in person or on the phone with:

SD1. A spouse (girl/boyfriend)

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD2. Your parents

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD3. Parents-in-law

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD4. Your children

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD5. Close family members

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD6. Neighbours that you feel you know quite well

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD7. Friends

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD8. People that you work with

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD9. Schoolmates

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD10. Members of a social or recreational group that you belong to (e.g. a sports team)

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

SD11. Members of a group with a religious affiliation

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

HEALTH AND SUPPORT

Drug dependency

DD1. Do you think your use of injection drugs is out of control?

0 Never/almost never
1 Sometimes
2 Often
3 Always/nearly always
55 Unsure
66 Not applicable
99 Refused to answer

DD2. Does the prospect of missing a fix make you anxious or worried?

0 Never/almost never
1 Sometimes
2 Often
3 Always/nearly always
55 Unsure
66 Not applicable
99 Refused to answer

DD3. Do you worry about your use of injection drugs?

0 Never/almost never
1 Sometimes
2 Often
3 Always/nearly always
55 Unsure
66 Not applicable
99 Refused to answer

DD4. Do you wish you could stop?

0 Never/almost never
1 Sometimes
2 Often
3 Always/nearly always
55 Unsure
66 Not applicable
99 Refused to answer

DD5. How difficult do you find it to go without using injection drugs?

0 Not difficult
1 Quite difficult
2 Very difficult
3 Impossible
55 Unsure
66 Not applicable
99 Refused to answer

HEALTH AND SUPPORT

Depression

DEP1. Have you recently been feeling unhappy and depressed?

0 Not at all;
1 No more than usual
2 More than usual
3 Much more than usual
55 Unsure
66 Not applicable
99 Refused to answer

DEP2. Have you recently been thinking of yourself as a worthless person?

0 Not at all;
1 No more than usual
2 More than usual
3 Much more than usual
55 Unsure
66 Not applicable
99 Refused to answer

DEP3. Have you recently felt that life is entirely hopeless?

0 Not at all;
1 No more than usual
2 More than usual
3 Much more than usual
55 Unsure
66 Not applicable
99 Refused to answer

DEP4. Have you recently been unable to concentrate on whatever you're doing?

0 Not at all;
1 No more than usual
2 More than usual
3 Much more than usual
55 Unsure
66 Not applicable
99 Refused to answer

DEP5. Have you recently been able to enjoy your day-to-day activities?

0 More so than usual
1 Same as usual
2 Less than usual
3 Much less than usual
55 Unsure
66 Not applicable
99 Refused to answer

DEP6. Have you recently tended to lose interest in your ordinary activities?

0 Not at all;
1 No more than usual
2 More than usual
3 Much more than usual
55 Unsure
66 Not applicable
99 Refused to answer

DEP7. Have you been feeling full of energy?

0 More so than usual
1 Same as usual
2 Less than usual
3 Much less than usual
55 Unsure
66 Not applicable
99 Refused to answer

DEP8. Have you recently felt that life isn't worth living?

0 Not at all;
1 No more than usual
2 More than usual
3 Much more than usual
55 Unsure
66 Not applicable
99 Refused to answer

HEALTH AND SUPPORT

Extraversion

Read: How would you describe yourself for the following list of common human characteristics. Using the first trait (adventurous) as an example, you can choose numbers from 1-9 with 1 being very unadventurous, 3 is moderately unadventurous, 7 is moderately adventurous, and 9 is very adventurous.

EV1. Unadventurous	1	2	3	4	5	6	7	8	9	Adventurous
EV2. Unassertive	1	2	3	4	5	6	7	8	9	Assertive **(willing to strongly state your opinion; confident and sure of yourself)**
EV3. Inactive	1	2	3	4	5	6	7	8	9	Active
EV4. Timid	1	2	3	4	5	6	7	8	9	Bold
EV5. Silent	1	2	3	4	5	6	7	8	9	Talkative
EV6. Unenergetic	1	2	3	4	5	6	7	8	9	Energetic
EV7. Introverted **(shy, withdrawn)**	1	2	3	4	5	6	7	8	9	Extraverted **(outgoing, sociable)**

HEALTH AND SUPPORT
Infection information for study participant

INF1. Have you ever been tested for hepatitis C? **If No, go to INF2, otherwise INF3.**

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

INF2. Can you tell me some of the reasons why you have not been tested for hepatitis C?

(please respond on separate answer sheet)

INF3. Have you ever been told that you are positive for hepatitis C? **If No, go to INF5, otherwise INF4.**

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

INF4. Who told you that you were positive for hepatitis C?

0 A nurse or doctor
1 A friend
2 Family member
3 Spouse/lover
4 Acquaintance/stranger
55 Unsure
66 Not applicable
99 Refused to answer.

INF5. Have you ever been tested for HIV (AIDS virus)? **If No, go to INF 6, otherwise INF7.**

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

INF6. Can you tell me some of the reasons why you have never been tested for HIV?

(Please respond on separate answer sheet)

INF7. Have you ever been told that you are positive for HIV? **If No, go to INF9, otherwise INF8.**

0 No
1 Yes
55 Unsure
66 Not applicable
99 Refused to answer

INF8. Who told you that you were positive for HIV (AIDS virus)?

0 A nurse or doctor
1 A friend
2 Family member
3 Spouse/lover
4 Acquaintance/stranger
55 Unsure
66 Not applicable
99 Refused to answer.

Read: Would you strongly agree, agree, disagree or strongly disagree with the following statements?

If INF3 was NO,
INF9. Becoming infected with hepatitis C is not one of my main concerns or worries.

0 Strongly agree
1 Agree
2 Disagree
3 Strongly disagree

If INF7 is NO,
INF10. Becoming infected with HIV is not one of my main concerns or worries.

0 Strongly agree
1 Agree
2 Disagree
3 Strongly disagree

HEALTH AND SUPPORT

Overall group norms

GN1.How many of your close friends talk about harm reduction and safe injection?

0 None
1 Very little/few
2 Less than half
3 About half
4 More than half
5 Almost all
6 All
55 Unsure
66 Not applicable
99 Refused to answer.

GN2. How many of your close friends or associates encourage you to inject drugs? **(verbal encouragement)**

0 None
1 Very little/few
2 Less than half
3 About half
4 More than half
5 Almost all
6 All
55 Unsure
66 Not applicable
99 Refused to answer.

GN3. How many of your close friends or associates would encourage you to always use clean needles? **(verbal encouragement)**

0 None
1 Very little/few
2 Less than half
3 About half
4 More than half
5 Almost all
6 All
55 Unsure
66 Not applicable
99 Refused to answer.

GN4. How many of your close friends would encourage you to always use your own equipment like cotton, rinse water, or cookers?

0 None
1 Very little/few
2 Less than half
3 About half
4 More than half
5 Almost all
6 All
55 Unsure
66 Not applicable
99 Refused to answer

GN5. How many of the IDU you know, try to always use clean needles for injecting drugs?

0 None
1 Very little/few
2 Less than half
3 About half
4 More than half
5 Almost all
6 All
55 Unsure
66 Not applicable
99 Refused to answer

GN6. How many of your close friends or associates encourage you to stop injecting drugs?

0 None
1 Very little/few
2 Less than half
3 About half
4 More than half
5 Almost all
6 All
55 Unsure
66 Not applicable
99 Refused to answer.

SOCIAL NETWORKS

1.) Network members:

Read: We are interested in the relationship between close personal contact and infectious diseases that are transmissible through used syringes, like hepatitis. We would like to ask you some questions about the people you normally associate with. We will not ask you for any information that could be used to identify those individuals and any information you provide to us will be confidential.

First, please think back over the last 30 days about the people with whom you have had more than casual contact. These would be people that you have seen or have spoken to on a regular basis. Most of these close contacts would be people such as friends, family, sex partners, people you inject drugs with, or people you live with.

Let's make a list of these people *(Interviewer - the maximum allowed on the list is 20 people. If the individual reaches 20 people ask them how many additional people they would be able to nominate and note their response on the answer sheet).*
Please use only initials, or some other identifier that will make sense to you such as a made up name. Please do not use their last names. We will use this list to make sure we know which individuals we are talking about. Remember that we are interested in people that you've had contact with in the last 30 days.

Interviewer: use the following prompts as needed, to help clients recall their associates.

People that you used drugs with in the last 30 days.
People who you had sex with during the last 30 days.
For subjects who are sex workers: list a maximum of 10 sex partners. If the name of a client is not known they can be listed as unknown1, unknown2, etc. If they have a regular sex partner(s) try to ensure that they are included on the list)
Friends, relatives or other individuals that you feel close to?
People you live with.
People you hang out with.

2.) Type of contact: *Interviewer: Once names are listed, please ask the participant the questions listed below and circle the appropriate letter by each name on the following page.*

Questions to ask regarding each of the network members listed on the following page:

1. **Which of these people has injected drugs in the last 6 months:** Enter Y (Yes) or N (No) or U (Unsure)

2. **Not including marijuana use, which of these people has smoked/snorted/inhaled drugs in the last 6 months**: Enter Y (Yes) or N (No) or U (Unsure)

3. **Which of these people has been a sex partner of yours in the last 6 months**: Enter Y (Yes) or N (No)

4. **What is the gender of each of these people?** Enter **M** Male, **F** female, **TM** transgender male, **TF** transgender female.

5. **What is the age of each of these people?**

6. **What is this person's relationship to you:** Enter **F** (family member), **L** (lover, spouse, girl/boyfriend), **R** (Friend), **C** (Acquaintance/Stranger).

7. **What is this person's ethnic group**: Enter the appropriate letter response

A. Caucasian/White;	I. Black-Caribbean
B. Chinese;	J. Other black
C. Filipino;	K. First Nations (treaty)
D. South-Asian	L. First Nations (non-treaty)
E. Other Asian	M. Metis
F. Latin American	N. Inuit
G. Middle Eastern	O. Other
H. Black-African	P. Unsure
	Q. Refused to answer

List of network members

Network member #	Network member Identifier	IV drug use	Smoke/Snort/ Inhale	Sex partner	Gender	Age	Relationship *(Co-workers, dealers, tricks, etc should be categorized as acquaintances unless person considers them a friend)*	Ethnic group
1		Y N U	Y N U	Y N	M F TM TF		F L R C	
2		Y N U	Y N U	Y N	M F TM TF		F L R C	
3		Y N U	Y N U	Y N	M F TM TF		F L R C	
4		Y N U	Y N U	Y N	M F TM TF		F L R C	
5		Y N U	Y N U	Y N	M F TM TF		F L R C	
6		Y N U	Y N U	Y N	M F TM TF		F L R C	
7		Y N U	Y N U	Y N	M F TM TF		F L R C	
8		Y N U	Y N U	Y N	M F TM TF		F L R C	
9		Y N U	Y N U	Y N	M F TM TF		F L R C	
10		Y N U	Y N U	Y N	M F TM TF		F L R C	
11		Y N U	Y N U	Y N	M F TM TF		F L R C	
12		Y N U	Y N U	Y N	M F TM TF		F L R C	
13		Y N U	Y N U	Y N	M F TM TF		F L R C	
14		Y N U	Y N U	Y N	M F TM TF		F L R C	
15		Y N U	Y N U	Y N	M F TM TF		F L R C	
16		Y N U	Y N U	Y N	M F TM TF		F L R C	
17		Y N U	Y N U	Y N	M F TM TF		F L R C	
18		Y N U	Y N U	Y N	M F TM TF		F L R C	
19		Y N U	Y N U	Y N	M F TM TF		F L R C	
20		Y N U	Y N U	Y N	M F TM TF		F L R C	

If the study participant nominates 20 network members, ask them how many additional people they could nominate and enter the number here:

Number of additional network members: _____

3.) **Interaction of network members:** *Interviewer: Following step 2), transfer the names of all of the network members to the interaction grid. For each person listed ask the subject to indicate which of the other individuals on the list that particular person knows.*

4.) Choose members: *Interviewer: Now transfer the names of all injection drug users onto the next part of the questionnaire shown below. If there are more than 5 IDU on the list place them, to a maximum of 5, on the questionnaire in the order the study participant placed them on the network member list.*

Network questions re each contact:
Interviewer: List the 5 network members chosen as per the above instructions and assign a code to each contact as follows (this information will be used by data entry to identify each contact of a given study participant.
a) List the first names or initials of the contacts chosen from the list under "initials/first name"
b) Enter the subject code from page 1 of the questionnaire on each of the "subject code" lines.
c) Assign a contact code (1 through 5) after the dash

Transfer the "initial/identifier" to a separate sheet of paper so you and the study participant can refer to it.

Initial/identifier	Subject code		Contact code (1-5)
_____	_____	—	_____
_____	_____	—	_____
_____	_____	—	_____
_____	_____	—	_____
_____	_____	—	_____

Example shown below:

Initial/identifier	Subject code		Contact code (1-5)
John	121	—	1
AJ	121	—	2
_____	_____	—	_____
_____	_____	—	_____
_____	_____	—	_____

Appendix 3 - Manitoba Health Report

The Winnipeg Injection Drug Use Social Network Study: Phase II

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Scientist, Cadham Provincial Laboratory

Assistant Professor, University of Manitoba

December, 2005

Manitoba

The Winnipeg Injection Drug Use Social Network Study: Phase II

Published: December, 2005

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3. Executive Summary

The data presented in this report originate from phase II of the **Winnipeg Injection Drug Use Social Network Study** (SNS II). This study is one of a series designed to examine the social networks of injection drug users (IDU) in Winnipeg and the importance and effects of social networks on an individual drug user's risk of infection, risk behaviours and harm reduction activities. This report focuses on the demographics and behaviours of the individuals interviewed and provides a current snapshot of the drug scene in Winnipeg.

- The study was based on administration of a quantitative questionnaire including a short number of open-ended questions and collection of a blood specimen for bloodborne pathogen diagnosis. A total of 435 individuals were enrolled in the study between December 2003 and August 2004.

- The last large scale study of IDU in Winnipeg was the **Winnipeg Injection Drug Epidemiology** (WIDE) study in 1998. The sociodemographic and socioeconomic profile of study participants was similar in SNS II compared to WIDE, suggesting that no major changes in the makeup of the IDU population in Winnipeg have occurred over the last 6 years.

- Bloodborne prevalence data (based on confirmed positives and negatives only) was 54% for Hepatitis C, 32% for Hepatitis B, and 7% for HIV. The prevalence for HIV is lower than the 12.6% found in WIDE. Although, the different study participant recruitment strategies must be kept in mind when comparing WIDE and SNS II, the comparison does suggest that HIV prevalence in this population has not shown a dramatic increase during the time between implementation of the two studies. Evidence for vaccination against HBV was found in 18% of study participants. Enhanced efforts to increase vaccination among IDUs should proceed as quickly as possible.

- Just over a quarter of the SNS II sample (28%) had moved to Winnipeg in the last year, reflecting the high mobility of this population. Thirty-two percent of these individuals moved here from other parts of Manitoba, 41% from the 3 western provinces, 25% from eastern Canada, and 2% from the US. The high mobility of IDU, both within and between provinces, highlights the importance of developing strong partnerships with other regional, provincial, and national agencies.

- For drugs used by injection, the most notable change since WIDE was the drop in cocaine as both a preferred drug and a most frequently injected drug. No one other drug has risen dramatically, rather it appears that several drugs have either increased in usage (i.e. talwin and ritalin, heroin, morphine) or have recently appeared (crystal methamphetamine, oxycodone). Despite the percentage drop in cocaine as either the most frequently injected or preferred drug of Winnipeg IDU, a large percentage of IDU (63.1%) still state that they have used this drug at some point in the past 6 months.

- After private residences (their own or a friend's place) hotels were the most common site of injection among IDUs. Almost half of all IDU had at some time in the past 6 months injected at hotels. Given that private residences are potentially a more difficult access

point for Public Health, these more public types of injection venues are important as potential Public Health contact points for the IDU population.

- Data were collected on various injection risk behaviours associated with bloodborne pathogen infection (giving away used needles, transferring drugs between syringes, using someone else's used syringe, how often those syringes were cleaned before reuse, frequency of cleaning used syringes, and using someone else's used drug preparation equipment). In most cases, the data suggest that many of these behaviours have become relatively rare, however, it was also clear that opportunity for disease transmission along these routes still occurs and these practices continue to occur in some circumstances even when an individual suspects a syringe has been used by someone infected by a bloodborne pathogen.

- Although it was clear that the majority of IDU found it easy to obtain new syringes, primarily either through needle exchanges or pharmacies, approximately 1/3 of IDU indicated a category of "somewhat easy" or lower in terms of their ability to readily access new syringes. Additional research and ongoing evaluation is required to ensure that programs are developed that appropriately target these IDU to improve their access to new syringes.

- For drugs used in a non-injection manner, several drugs, such as crack or cocaine, were nearly as commonly used as alcohol and marijuana. The emergence of the use of crystal methamphetamine was also evident. The most common drugs smoked, inhaled, or snorted were crack, cocaine, crystal methamphetamine, gasoline/solvents, and heroin. The most common response regarding frequency of sharing or re-using straws or pipes for smoking drugs was "always". Many participants had also indicated they had had cuts or burns on their lips or in their mouth in the last 6 months due to crack smoking. This combination of sharing equipment/supplies and breaks in the integrity of the skin increases the risk for transmission of bloodborne pathogens.

4. Introduction

This study represents phase II of the **Winnipeg Injection Drug** (SNS II); one of a series of studies on the social networks of injection d g and the importance and effects of social networks on an individual risk behaviours and harm reduction activities. In collecting this type nt of information must also be collected on the demographics and beha ell as their respective social networks. Much of this individual data t for understanding the drug scene in Winnipeg. This report focuses ch of the social network data are also of interest, but it will be publishe will focus on specific research questions and hypotheses. Those repo peer-reviewed literature. Notices and copies of these publications wi come available to the various agencies and clinics in Winnipeg which fo use.

As noted above, the purpose of the present report is to focus on a s and associated drug use behaviours, essentially providing a current drug scene in Winnipeg in 2004. Specifically, IDU demographics, non g use behaviours, risk behaviours, and needle exchange use are highlig s are compared to those from the **Winnipeg Injection Drug Epider** d out in 1998 (1) to identify and track trends or changes in the Winn ne of data collection for that study.

4.1 Overall Study Objectives

The overall objectives of SNS II, as presented to our funding a es of Health Research are as follows:

1. Analyze the social context of syringe sharing among IDU.
2. Correlate social network variables with the seroprevalenc IV.
3. Analyze the molecular epidemiology of HCV within soci
4. Correlate immune system status with social behavioural d
5. Construct and analyze sociometric networks of IDU

5. Methods

5.1 Study Setting

SNS II was carried out within the City of Winnipeg, Manit the provincial capital of Manitoba and the largest urban pop ince (approximate population of 670,000 out of a provincial populatio n).

5.2 Study Rationale and Origin

Phase I of the Winnipeg Social Network IDU study originat ipeg Regional Health Authority was interested in gathering data on U in Winnipeg. This request followed the successful investigation s of STD cases and contacts by Drs. John Wylie and Ann Jolly and se I

was meant to be largely exploratory; primarily for the purpose of determining whether Winnipeg IDU were willing to provide information on their social networks and to gather data that would allow specific testable hypotheses to be formulated for incorporation into future studies.

Both of these goals were clearly met in phase I. IDU in Winnipeg were willing to discuss their connections with other people, as long as it was done in a confidential, anonymous manner. It was also clear from preliminary analysis of these data that several aspects of Winnipeg IDU social networks could potentially contribute to the transmission of bloodborne and sexually transmitted pathogens. It was these latter findings which led to the hypotheses and specific data items incorporated into the SNS II proposal and its successful funding by the Canadian Institutes of Health Research.

5.3 Subject Recruitment

The enrollment criteria for possible inclusion in SNS II was a history of injecting drugs in the *6 months* prior to interview. This differed from WIDE where the drug use criteria was *ever* having injected illicit drugs. WIDE also enrolled the majority of its study participants through referral of potential study subjects by various agencies/clinics working with IDU. In contrast, SNS II enrolled almost entirely through word-of-mouth advertising and self-referral to the study nurse, relying on communication structures within the IDU population. Additionally WIDE gathered data over a 12 month period *vs.* 9 months for SNS II. Given that study recruitment and deployment were not identical, any comparisons between SNS II and WIDE should be considered within the context of these differences. A random sample of IDU is not possible, and different trends may reflect true differences (e.g. a change inherent to the IDU population or a change brought on by implementation of or changes to a program since WIDE), or simply reflect the different populations accessed by a given sampling strategy.

Regardless of the underlying reason, in areas where the two studies have produced different results, caution should be considered before making any major program or policy decisions based on that change. Rather, differences should be interpreted either as true changes or indications that our knowledge or beliefs of the IDU population with respect to a given variable, may not be as accurate as thought. In contrast, when the two studies do agree, it helps to bolster our ideas that, for those variables, there is a greater likelihood that the characteristics of the IDU population in Winnipeg have been accurately represented.

During the first few months of SNS II, there was overlap with another study in Winnipeg, the **Enhanced STD surveillance in Canadian Street Youth Study, Phase IV.** Margaret Ormond was the study nurse for both studies, and as such, if Street Youth participants met the SNS II criteria, she invited them to join the study. She also established a presence in different neighbourhoods, to establish connections to the community and spread word of the study in an informal, conversational way.

Most individuals were interviewed at their homes. Care was taken to ensure that the circumstances of the interview allowed for a private, confidential interview to take place. Frequent use was also made of neutral places, such as Sunshine House, if participants preferred or were more comfortable with a venue of this type.

5.4 Data Collection

SNS II relied primarily on a quantitative questionnaire format with a small number of short answer open-ended questions. The questionnaire consisted of two main parts – Part I questions pertained primarily to the study participant themselves followed by Part II where questions pertained to that person's social network.

Part I consisted of the following main sections:

i) demographics
ii) individual drug behaviours
iii) needle sources
iv) binges
v) smoking, inhaling, or snorting drugs
vi) sexual behaviours
vii) social support
viii) social diversity
ix) drug dependency
x) depression
xi) extraversion
xii) infection status knowledge
xiii) overall group norms

Many of the latter sociobehavioural sections (e.g. depression, extraversion) were designed specifically to answer certain questions pertaining to IDU behaviours and will not be discussed further in this report.

Part II consisted of the social network portion of the questionnaire. In this section, study participants were asked to think back over the last 30 days about the people with whom they had had more than casual contact. Prompts included people that they had used drugs with; people that they had sex with; friends, relatives, or other individuals they feel close to; people they lived with; people they hung out with.

Using initials or other anonymous identifiers, participants were asked to list a maximum of 20 members of their social networks (referred to as contacts or network members in this report). They were asked basic questions about each of these people including identifying which were injection drug users; which used drugs in a non-injection manner; which were sex partners of the participant; and their gender, age, ethnicity, and relationship to the study participant.

Next the questionnaire focused on the network contact members that were IDU (as reported by the study participant). A series of detailed questions were asked about each of these individuals to a maximum of 5 IDU. If more than 5 IDU were on the list they were chosen in the order that the study participant listed them originally. The detailed questions were formatted as shown in the example below:

CD4. How frequently would you say you have contact with [person]?

	Network Member #				
	1	2	3	4	5
0 Daily					
1. 2-4 times per week					
2. Once a week					
3. 1-3 times per month					
4. Less than once per month					
55 Unsure					
66 Not applicable					
99 Refused to answer					

In this manner the study participant was asked a single question, but could provide different answers corresponding to each of the IDU network members. This part of the questionnaire contained the following sections:

i) contact demographics
ii) contact injection drug behaviours
iii) contact non-injection drug behaviours
iv) characteristics of the relationship (i.e. between the study participant and a given contact)
v) relationship norms and expectations
vi) initiation and demonstration of injection

In addition to questionnaire administration, participants were asked if they would provide a blood specimen for determination of hepatitis C (HCV), HIV, and hepatitis B (HBV) infection status and whether participants had been vaccinated against HBV. Testing was conducted on an ongoing basis to allow study participants access to their test results in a timely manner. Testing was done based on a study participant's study code, therefore, when information on previously undiagnosed infections were given to participants, they were informed that they would have to present to primary health care for retesting and follow-up. A referral process to clinical/treatment sites had been established by the study nurse as part of the start-up phase of the study. Additionally, clinical questions were answered by the study nurse as comprehensively as possible, and resource material was frequently left behind with the participant.

Provision of results by return follow-up appointments was generally not successful. However, more success was obtained by suggesting that participants re-call the study nurse when they were ready to receive test results.

An honorarium of $40.00 was provided to study participants for taking part in any aspect of the study (questionnaire and/or specimen collection). In practice, 88% of study participants who completed the questionnaire, also provided a blood specimen. The remainder either refused to provide a specimen or were unable to provide a specimen (e.g. due to damaged veins).

As part of their participation, a short summary sheet of the main findings/aspects of Phase I of the study was made available to study participants. This provision of results appeared to be well-received by study participants and helped in fostering continuing community involvement in Phase II.

6. Results and Discussion

6.1 Demographics

A total of 435 study participants were enrolled (in the various tables presented in this report, total participants may number less than 435 depending on the amount of missing data for a given question). This target enrollment number was based on a sample size calculation required for one of the research questions included within the CIHR funding proposal. This sample is slightly smaller than the sample size of 608 used in WIDE, however, in both cases, the relatively large number of people interviewed provides for a robust cross-section of the IDU population in Winnipeg. Participants in SNS II ranged in age from 16 to 64 with representation across all age groups (Table 1). The median age was 35 (males, 36; females, 35).

Table 1. Frequency distribution of study participant age

Age group	Number of study participants	Percent
15-19 years	27	6.24%
20-24 years	53	12.24%
25-29 years	55	12.70%
30-34 years	67	15.47%
35-39 years	79	18.24%
40-44 years	79	18.24%
45-49 years	42	9.70%
>50 years	31	7.16%
total	433	100.00%

Fifty-six percent (247) of the participants were male, 43% (186) were female, and 1.4% (6) were transgender female (biological male). With respect to ethnicity, 47% (204) self-identified as First Nations (either treaty or non-treaty), 34% (149) as Caucasian/white, 15% (68) as Métis, and 2.7% (12) as "other" (Latin American, Mid-East, Caribbean-black, Inuit). Forty percent (174) of the participants were born in Winnipeg, 30% (132) outside Winnipeg, but within the province of Manitoba, 26% (113) were born in other Canadian provinces or territories, while the remaining 3% (14) were foreign-born. The majority of participants had dropped out of school (35% [150] before grade 9; 34% [148] between grades 10 - 12). Eleven percent (48) graduated grade 12, 16% (69) pursued additional educational activities after grade 12 (e.g. university, community college), while 4% (18) were currently pursuing their grade school education.

Just over a quarter of our sample (28%) had moved to Winnipeg in the last year, reflecting the high mobility of this population. Thirty-two percent of these individuals moved here from other parts of Manitoba, 41% from the 3 western provinces, 25% from eastern Canada, and 2% from the US. The high mobility of IDU, both within and between provinces, highlights the importance of developing strong partnerships with other regional, provincial, and national agencies.

The primary source of income for the majority of participants (65% [278]) was some form of support (i.e., government support in the form of welfare, employment insurance, etc. or support

from family or friends). Twenty-three percent (97) had full- or part-time employment, while the remaining 13% (56) listed either sex trade (12, 2.8%), dealing drugs (10, 2.3%) or stealing, boosting (the selling of stolen goods), or panhandling (34, 7.9%), as their main source of income.

Although a relatively small number of individuals relied on sex trade, drug dealing, or other forms of illicit activity as their main source of income, many partially relied on these activities to supplement their income. Table 2 shows this latter data and also illustrates the percentage of people reporting their supplementary types of income from all other sources. This differs from the data in the preceding paragraph where only main income sources are discussed. The majority of participants were either living in their own house or apartment (48%, 207), a friend's house or apartment (12%, 52) or a family member's house or apartment (12%, 50). The remainder were living in a hostel, rooming house, or some other form of shelter (13%, 57), in a hotel (9%, 39), or on the street or in a vehicle (6%, 27).

Table 2: All types of supplementary income sources indicated by study participants (Corresponding question on questionnaire - Over the last year what other ways did you get money to live on? – more than one response allowed per participant)

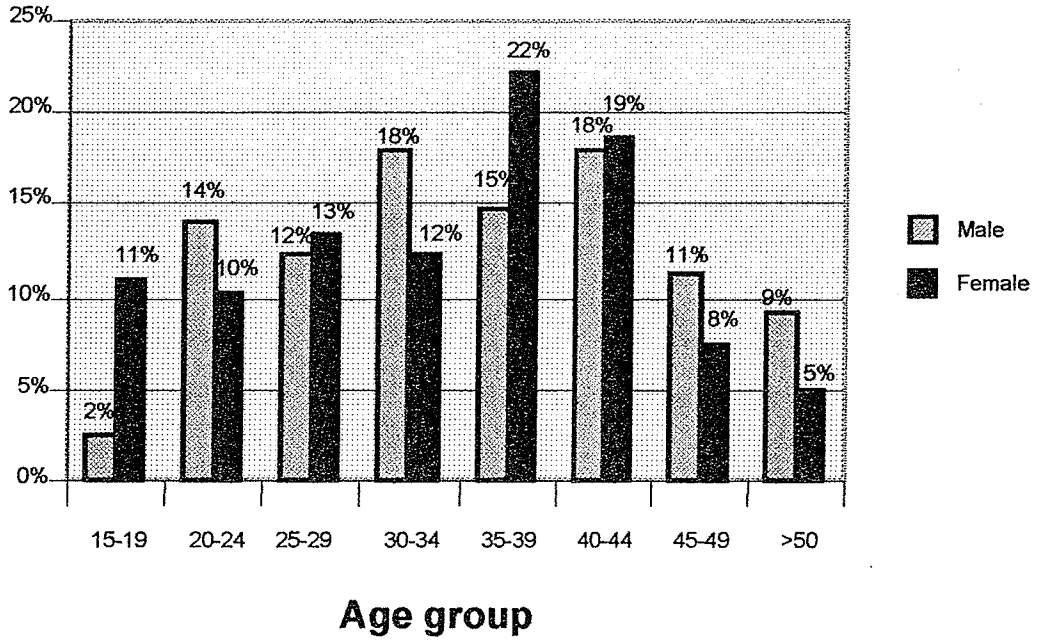
Income source	Number of participants indicating income from this source	Percent
regular work (full, part time or contract)	165	38.11%
welfare, EI, pension or other government support	117	27.02%
money from family/friends	235	54.27%
sex trade/prostitution	83	19.17%
dealing or doing drug runs	208	48.04%
Panhandling	71	16.40%
Stealing	137	31.64%
Boosting (selling of stolen goods)	129	29.79%
Other	41	9.47%
Total number of participants providing responses for this question - 433		

The male/female gender and ethnicity ratios in SNS II and WIDE were very similar, suggesting the overall demographic characteristics of the population have not changed substantially over the past several years nor were they affected by the different sampling strategies in the two studies. Additionally, the gender ratio stratified by ethnicity is also very similar in SNS II and WIDE. In both studies there was a 3:1 ratio of males to females for Caucasians, while the ratio was near equality in both studies for aboriginals (WIDE was 47% male: 52% females, while SNS II was 44% males: 56% females for First Nations and 53% males: 47% females for Métis).

In contrast to the clear trend towards young IDU being female in WIDE (in WIDE the three youngest age groups [15-19, 20-24, and 25-29] were each dominated by females), this trend was less clear in the SNS II data (Fig. 1). The youngest age group was heavily skewed towards females, however, the 20-24 year age group was skewed towards males, as was the 30-34 year age group. The 25-29 year age group was close to evenly split between males and females. This discrepancy between SNS II and WIDE is also reflected in the differing percentages of male and

female IDU less than 30 years of age (SNS II - 34% female, 29% male; WIDE – 41% female, 25% male). Although it is clear that many young IDU are female, the greater tendency for young IDU to be female, suggested by WIDE, is not as evident in SNS II. The trend may have begun near the time when WIDE was implemented, however, the pattern is much less clear today.

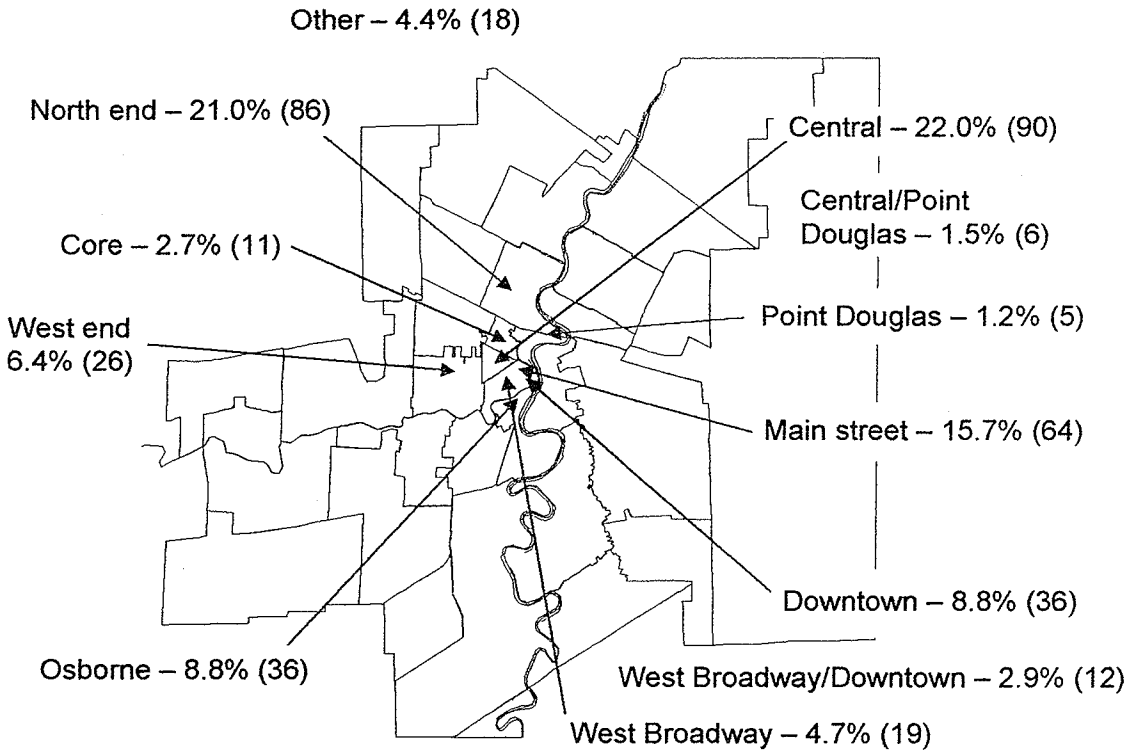
Fig. 1. Age and gender distribution of SNS II study participants.



The socioeconomic indicators from the two studies are generally similar. Government support from welfare, employment insurance, and other similar sources, continues to be the main income source for most IDU. The percentage of individuals citing sex trade and other illegal activities as main income sources in SNS II appears lower than WIDE (13% vs. 27%), while a greater percentage of IDU cite regular employment as their main income in SNS II than WIDE (22% vs. 11%). These differences could easily reflect the different sampling strategies as, for instance, sex trade workers, may be more likely to have enrolled in WIDE due to its focus on community agencies as enrollment sites. The percentage graduating high school was slightly higher in WIDE (SNS II – 27% vs. WIDE – 35%)

In SNS II, questions were also asked about a person's place of residence in the city, and where they normally hang out. Figure 2 illustrates the various places in the city where people say they typically hang out. Several individuals could not be definitely placed in a specific area based on their answers and some combination areas (e.g. Central/Point Douglas and West Broadway/Downtown) are included on the map. The three most common areas were the North End, the Central area of Winnipeg, and the Main Street strip, followed by Osborne and the Downtown area. The "other" area indicated at the top of the map is a catch-all category and, in effect, represents anyone who hung out in Winnipeg, but not in one of the designated areas on the map.

Figure 2. Areas where IDU say they "hang out".



6.2 Prevalence Data

Prevalence data for HCV, HIV, and HBV (both infection and vaccination status) was determined using the blood specimens IDU provided (Table 3).

Table 3. Prevalence data for HCV, HIV, HBV, and HBV vaccination status of study participants. HBV prevalence was based on the presence of antibodies to the core protein of HBV. HBV vaccination is based on the presence of antibodies to HBV surface antigen only.

Result	Number of participants	Percent (based on positive, negative and indeterminant specimens only)
HCV		
Positive	209	54.15%
Negative	175	45.34%
not determined or indeterminant	2	0.52%
Specimen not available*	49	
HIV		
Positive	28	7.39%
Negative	351	92.61%
not determined or indeterminant	56	
HBV		
Positive	122	31.77%
Negative	261	67.97%
not determined or indeterminant	1	0.26%
Specimen not available*	51	
HBV vaccination		
Yes	68	17.85%
No**	293	76.90%
specimen not available*	54	
Low***	20	5.25%

* specimens were either not available due to refusal to provide a specimen or the inability to collect sufficient specimen from some individuals.
** "no" indicates both people who are unvaccinated and uninfected and those infected by HBV.
*** "low" indicates people who had weak positive results against surface antigen and could represent either false positive reactions or a low positive vaccination status

Prevalence data (based on positive, negative, and indeterminant specimens only) indicate that 54% of IDU have experienced infection with HCV, 32% have experienced an HBV infection, while HIV has infected 7% of the study participants. For the latter, it is notable that this figure is lower than the 12.6% found in WIDE. Although, the different recruitment strategies must be kept in mind when comparing WIDE and SNS II, the comparison does suggest that HIV prevalence in this population has not shown a dramatic increase during the time between implementation of the two studies. Additionally, although the prevalence of HCV is clearly high, it is lower than seen in some other Canadian cities based on the pilot phase of the I-track National Surveillance Project (6) and there remain many opportunities for the prevention of transmission between infected and uninfected IDU. HBV has not spread to the extent that HCV has in this population, however, it is also clear that there are relatively few IDU that have been vaccinated against this infectious agent.

Enhanced efforts to increase vaccination among IDUs should proceed as quickly as possible to reduce or prevent further infection and transmission of HBV in this population. It should be noted that depending on the infection status of those individuals who refused testing or who were unable to provide a specimen, the true prevalence of the bloodborne pathogens noted above could be higher.

Questions were also posed to the study participants with respect to whether they had been previously tested for HCV or HIV. Prior to enrollment in the study, 81 (18.8%) and 70 (16.4%) study participants had not been tested for either pathogen. Although it is encouraging that a large majority of IDU had been tested and were aware of their infection status, further efforts to increase testing is warranted. Awareness of infection status is important not only to ensure an individual is receiving proper care, but also due to the existence of altruistic behaviour on the part of IDU to prevent further transmission from infected to uninfected individuals, that has been noted in other locales (7, 8). Whether altruistic behaviours of this type occur in Winnipeg IDU has not yet been studied.

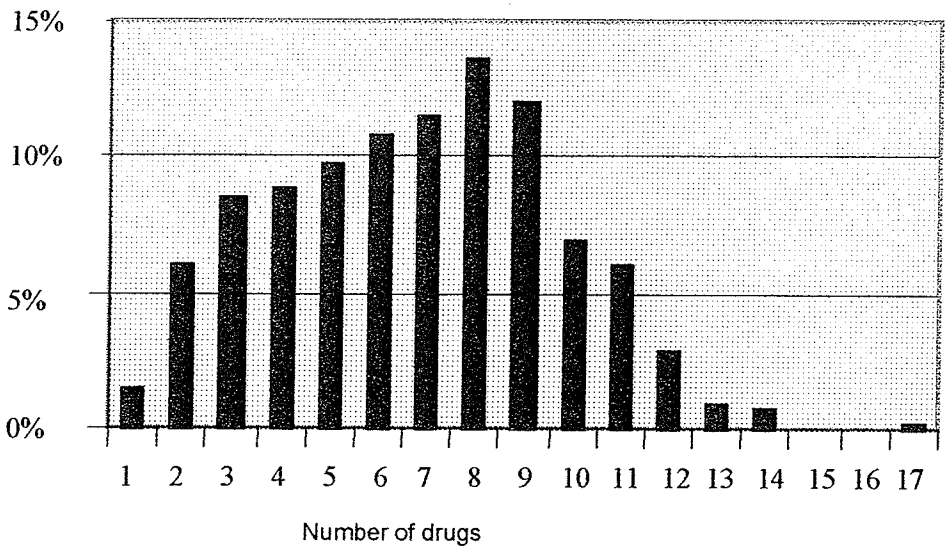
6.3 Non-injection Drug Use

Table 4 illustrates which drugs participants have used in a non-injecting manner. Several drugs, such as crack or cocaine, are nearly as commonly used as alcohol and marijuana. The emergence of the use of crystal methamphetamine is also seen, although by a relatively small number of participants. Fig. 3 shows the number of different drugs study participants use by routes other than injecting (alcohol and marijuana have been excluded from the data used in Fig. 3), and illustrates the frequency of poly-drug use.

Table 4. Drugs used in a non-injecting manner by study participants. Listed in order of most to least common (with exception of "other" drugs).

Drugs used without injecting	Number of participants indicating use of drug	Percent
Alcohol	388	89.61%
Marijuana	336	77.60%
Crack	332	76.67%
Cocaine	296	68.36%
downers/tranquilizers	290	66.97%
Tylenol 3	279	64.43%
Barbiturates	235	54.27%
Painkillers	206	47.58%
Demerol/morphine/opium	189	43.65%
Mushrooms	107	24.71%
methadone unprescribed	89	20.55%
Amphetamines	86	19.86%
crystal methamphetamine	71	16.40%
methadone prescribed	65	15.01%
Acid	54	12.47%
gasoline/solvents	50	11.55%
Heroin	43	9.93%
Ecstasy	31	7.16%
PCP/angel dust	21	4.85%
other drug	51	11.78%
Total number of participants providing responses for this question - 433		

Figure 3. The number of non-injection drugs used by study participants. The percent data reflects the percent of study participants using a given number of drugs. Alcohol and marijuana have been excluded from this data.



A more specific set of questions was also asked on smoking, inhaling, or snorting drugs in the 6 months prior to interview. Ninety-four percent of respondents answered "yes" for this question. Excluding marijuana and those individuals who use only marijuana, the most common drugs used in this manner were crack (328 participants, 80%); cocaine (227, 55.5%), crystal methamphetamine (87; 21.3%) gasoline/solvents (47; 11.5%) and heroin (29, 7.1%). All other types of drugs were indicated by less than 3% of participants. The most common response to the question, "Excluding marijuana use, in the past month, how often did you smoke/inhale/snort drugs?" was "once in a while, not every week" (135 participants, 35.3%), while 64 (16.7%) participants indicated they used drugs in this manner on a daily basis. In addition to private residences, other common places to smoke or snort drugs were on the street, (152 participants, 39.7%); hotels (144, 37.6%); vehicles (127, 33.2%); rooming/boarding houses (77, 20.0%) and shooting galleries (49; 12.8%).

Participants were asked about their re-use or sharing of straws/pipes. Two hundred and eighty-three (76.3%) of the 371 participants who are smoking drugs other than marijuana, indicated they do share equipment. Frequency choices for this behaviour were "hardly ever" (26 participants, 9.2%); "sometimes" (79, 27.9%); "frequently" (64, 22.6%); and "always" (114, 40.3%). Of the 328 participants who indicated they smoked crack, 99 (30.6%) said that, in the prior 6 months, they have had cuts or burns on their lips or in their mouth due to crack smoking. The frequency with which sharing of straws/pipes occurs and the frequency with which burns occur, highlights the need for ongoing distribution of crack kits to this population to potentially help reduce pathogen transmission via this route.

6.4 Injection Drug Use Behaviours

The median and mean age of first injection was 19 and 21 years, respectively. Seventeen percent of participants indicate they inject on a daily basis, 29% at least one or more times per week. Fifty-seven percent binged on drugs in the 6 month period prior to their interview date. The most common age of first injection was between 13 and 17 (152 participants; 35%). Twenty participants (4.6%) began injecting between the ages of 8 and 12.

The most frequently injected drugs (the one drug that a study participant most frequently injects) and preferred injection drugs (the one drug that a study participant would prefer to inject if availability/price was not an issue) are shown in Tables 5 and 6, respectively. The most notable change since WIDE was the drop in cocaine as both a preferred drug and a most frequently injected drug (cocaine was a preferred drug of 60% of respondents in WIDE). No one other drug has risen dramatically in the place of cocaine, rather it appears that several drugs have either increased in usage (e.g. Talwin and ritalin, heroin, morphine) or have recently appeared (crystal methamphetamine, oxycodone).

Table 5. Preferred injection drug as indicated by study participants (only one choice allowed per study participant).

Drug name	# of participants	Percent
cocaine	168	38.80%
talwin & ritalin	87	20.09%
heroin	57	13.16%
Morphine	39	9.01%
crystal methamphetamine	19	4.39%
Crack	10	2.31%
Oxycodone	8	1.85%
Dilaudid	7	1.62%
Other	31	7.16%
Unsure	6	1.39%
not applicable	1	0.23%
Total	433	100.00%

Table 6. Most frequently injected drug as indicated by study participants (only one choice allowed per study participant).

Drug name	# of participants	Percent
cocaine	157	36.43%
talwin & ritalin	103	23.90%
Morphine	70	16.24%
Crack	24	5.57%
crystal methamphetamine	22	5.10%
heroin	14	3.25%
Oxycodone	7	1.62%
Dilaudid	11	2.55%
Other	20	4.64%
Unsure	2	0.46%
not applicable	1	0.23%
total	431	100.00%

Table7 shows, in total, the range of drugs participants have injected in the last 6 months. For this question participants were allowed to select as many drugs as were applicable.

Table 7. Total overall usage of injection drugs by study participants. Corresponding question on questionnaire – Which drugs have you injected in the last 6 months.

Drugs injected in the last 6 months	Number of participants indicating use of this drug	Percent
Cocaine	273	63.05%
Morphine	156	36.03%
talwin & Ritalin	140	32.33%
crack/rock cocaine	105	24.25%
Dilaudid	71	16.40%
Heroin	43	9.93%
Crystal methamphetamine	43	9.93%
Methadone	26	6.00%
ritalin alone	23	5.31%
Oxycodone	17	3.93%
Amphetamines	14	3.23%
heroin & cocaine	12	2.77%
heroin & other drugs	3	0.69%
Barbiturates	3	0.69%
PCP	1	0.23%
"other"	33	7.62%

Despite the percentage drop in cocaine as either the most frequently injected or preferred drug of Winnipeg IDU, a large percentage of IDU (63.1%) still state that they have used this drug at some point in the past 6 months. Morphine is the second on the list, despite Talwin/Ritalin being the second choice for most frequently injected drug and second on the list of preferred drugs of IDU (Tables 5 and 6). Again, as above, the emergence of crystal methamphetamine and, to a lesser extent, oxycodone on the Winnipeg scene is evident. Using the data of Table 7, the number of drugs injected by IDU was calculated. In comparison to the poly-drug use of Fig. 3 for using drugs in a non-injection manner, most IDU indicate they have only used one injection drug in the past 6 months (189 participants, 43.7%), with a maximum of 9 drugs indicated by one study participant.

Questions were asked regarding the types and number of places where drugs were injected (Tables 8 and 9).

Table 8. Types of places most frequently used as a venue for injecting drugs. Only one choice was allowed per study participant (Over the last 6 months, at what type of place have you most frequently injected?).

Place of injection	Number of participants	Percent
at your own house or apartment	180	41.67%
at a friend's house or apartment	154	35.65%
Hotel	28	6.48%
on the street	24	5.56%
shooting gallery	18	4.17%
at family member's house or apartment	13	3.01%
Other (e.g. rooming/boarding house, vehicles)	15	3.47%
Total	432	100.00%

Table 9. All types of places injected at by study participants. More than one choice allowed per study participant (Questionnaire question - Over the past 6 months, what types of places have you injected drugs?)

Place of injection	Number of study participants choosing a given category	% yes
at friend's house	309	71.36%
at own house	248	57.27%
Hotel	176	40.65%
rooming/boarding house	134	30.95%
on the street	126	29.10%
Vehicle	106	24.48%
public washroom	84	19.40%
shooting gallery	67	15.47%
at family's house	62	14.32%
empty house	61	14.09%
hostel/shelter	11	2.54%
recovery house	9	2.08%
jail or prison	4	0.92%
Other	40	9.24%
Total number of participants providing a response for this question - 433		

In both cases, after private residences (own or friend's place) hotels were the most common site of injection (most frequently used by 28 [6.5%] participants and used overall by 176 [40.7%]). Public places including the street, public washrooms, and vehicles are also commonly used by many IDU. Given that private residences are potentially a more difficult access point for Public Health, it is notable that almost half of all IDU have at some time in the past 6 months injected at a hotel. Given the importance of hotels in terms of both a potential site of pathogen transmission

and as a Public Health contact point, analysis of hotel data is currently underway and is expected to be the first report published subsequent to the present report.

6.5 Risk Behaviours

Several questions were asked on various risk or harm reduction behaviours to provide a current overall picture of these activities in Winnipeg. The vast majority of IDU in Winnipeg have indicated they have not given away a used needle to another IDU in the past 6 months (369 participants, 85.4%). Fifty-seven (13.1%) people have done this occasionally or sometimes in the past 6 months, while a small minority indicated they usually or always give away their needles (3 people, 0.9%).

Questions were also asked regarding the frequency of transfer of prepared drugs between syringes. In this behaviour, drugs are prepared in one user's syringe and transferred to another user's syringe. Although the syringes themselves are not shared, if the drug preparation syringe has been previously used for injection, it could be contaminated with a pathogenic agent. Therefore, the drugs being transferred could be likewise contaminated and potentially result in pathogen transmission. As above, this is a relatively rare behaviour with 356 participants (82.4%) indicating they have never done this in the previous 6 months. Sixty-one people (14.1%) have done this occasionally or sometimes, while 7 people (1.6%) did this usually or always.

Although the majority of IDU (60.4%) have either used someone else's used syringe (or were unsure whether they had) at some point in the past, the majority have said they did not use anyone else's used syringe in the previous 6 months (337, 78.2%). However, this still leaves almost a quarter of study participants who either indicated they have definitely used someone else's used syringe in the previous six months (72, 16.7%) or weren't sure whether they had engaged in this behaviour (22, 5.1%).

Of the 72 study participants who indicated they had definitely used someone else's used syringe in the previous 6 months, 65 provided a response to how often they cleaned those needles first before use. Seventeen (26.15%) indicated they never or hardly ever cleaned them first, 19 (10.77%) indicated "sometimes" while 45 (60%) indicated "frequently" or "always". Two were unsure how often they cleaned needles previously used by someone else. Also, it is interesting to note that of these 72 study participants, 28 (43.1%) knowingly used a syringe after someone they suspected or knew to be infected with HIV and/or HCV had used it first. Another 7 (10.8%) were unsure if the person from whom they received the used syringe was infected with either pathogen.

The use of someone else's used equipment, including cookers, rinse water, cotton, etc. is not practiced by the majority of IDU, however, it is clearly more common than the reuse of used syringes. Two hundred and seventy-four study participants (63.4%) indicated they had not done this in the previous 6 months, while "occasionally", "sometimes", "usually", and "always" was chosen by 67 (15.5%), 34 (7.9%), 23 (5.3%), and 18 (4.2%) study participants, respectively. Sixteen (3.7%) were unsure whether they had used someone else's used equipment.

This discrepancy in the frequency of sharing syringes vs. sharing equipment is also reflected in the different beliefs in the risk posed by syringes vs. equipment. Three hundred and eighty-four (88.9%) and 376 (87.0%) IDU believe that transferring drugs between syringes poses a risk for HCV and HIV transmission, respectively. This number drops to 334 (77.5%) and 306 (71.0%)

when asked if sharing drug preparation equipment poses a risk for transmission of these same two pathogens.

In most cases the data suggests that sharing behaviours or the re-use of someone else's syringes/equipment has become relatively uncommon as IDU take steps to protect themselves. However, it is also clear that opportunity for disease transmission along these routes still does occur and these practices continue to occur in some circumstances even when an individual suspects the needle has been used by someone infected by a bloodborne pathogen.

6.6 Use of Needle Exchanges

The introductory question for this section was, "In the past six months, have you exchanged needles or gotten new needles at a needle exchange?" Two hundred and seventy-six (63.9%) study participants responded affirmatively for this question. Two hundred and seventy-four participants provided information on how many needles they usually obtained in this manner. The two most common responses were "most" (113, 41.1%) and "less than half" (81 (29.5%). Forty-one (14.9%) responded "all", while 39 (14.2%) said "about half". A separate question was also asked on where individuals in general obtained syringes as well as the specific name of the needle exchange site they had obtained syringes from (Table 10).

Table 10. Sources of new syringes accessed by study participants in the previous 6 months. As many choices as applicable were allowed.

Syringe sources	Number of participants indicating they had used this source	Percent
friends/partners/family	278	64.35%
pharmacy/drugstore	275	63.66%
street connections	207	47.92%
other needle exchanges*	138	31.94%
Dealer	60	13.89%
someone on the street	59	13.66%
Nine Circles	45	10.42%
Sage House	35	8.10%
Sunshine House	32	7.41%
from other cities	30	6.94%
nurse/doctor/hospital	25	5.79%
shooting gallery owner	20	4.63%
found on the street	2	0.46%

Total number of participants providing responses for this question – 433
* here "other" implies needle exchanges other than the ones specifically listed above.

When asked to choose the one site where they obtained the majority of their new syringes from, most IDU selected "Street Connections" (140, 32.6%), followed by pharmacy/drugstore (125, 29.1%), friends/partners/family (93, 21.7%), needle exchanges other than Street Connections (62, 14.5%), and "someone on the street" (9, 2.1%). The majority of individuals indicated it was "very easy" to obtain new needles (282, 65.3%), followed by "somewhat easy" (85, 19.7%), "somewhat difficult" (40, 9.3%), "very difficult" (22, 5.1%), and "unsure" (3, 0.7%).

Although it is clear that the majority of IDU find it easy to get new syringes, primarily either through exchanges or pharmacies and drugstore, approximately 1/3 of IDU indicated a category of "somewhat easy" or lower in terms of their ability to readily access new syringes. Additional research and analysis is required to delve into the details of whom these individuals are and why they have some difficulty in obtaining syringes. Power relationships, in which some IDU may depend on others to obtain needles for them, may play a role in ease of access and also risk, as these "dependent" IDU may have poorer access to harm reduction educational efforts available to exchange attendees.

7. Summary

This report highlights the current Winnipeg IDU scene in 2004 with respect to various demographic and injection and non-injection drug behaviours and provides an update of the data collected during WIDE, the last major IDU study to have taken place in Winnipeg since 1998. The data presented here were collected as part of Phase II of the Winnipeg IDU Social Network Study. In general, with respect to many sociodemographic and socioeconomic variables, the IDU population in Winnipeg appears quite similar to that represented in WIDE. Some prevalence data is encouraging given that HIV does not appear to have increased since WIDE and approximately half of the IDU interviewed have not been infected by HCV. However, work still needs to be done to increase HBV vaccination rates. The emergence of new drugs, including crystal methamphetamine and oxycodone, is also documented in our results. The high frequency with which equipment used for smoking drugs is shared and the relatively large number of individuals who report burns or cuts on their lips or in their mouth from crack smoking suggests opportunities exist for potentially reducing transmission of pathogens via this route. With respect to injection drug use, there appears to have been a decrease in the frequency of cocaine use with many other drugs having risen in prominence (although cocaine is still the most common drug used). The central importance of Winnipeg hotels as places of injection is also clear, with almost half of all IDU interviewed having injected at a hotel in the last six months. Data on risk behaviours suggest that many IDU are taking steps to protect themselves from infection, however, some IDU are still placing themselves at risk through the re-use of syringes and/or drug-preparation equipment. Additional data analysis now ongoing will provide further insights into the correlates associated with infection by bloodborne pathogens or risk behaviours such as syringe sharing within this population.

8. References

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