Investigating Biological and Social Factors Influencing the HIV Epidemic in Manitoba

by

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Abstract

Taking into account that both biological and social factors influence the dynamics of HIV disease, the components of this thesis aim to employ an interdisciplinary approach to exploring host factors which may have important consequences for HIV risk and disease progression in Manitoba and be relevant to Aboriginal populations. Two separate projects were undertaken and are presented within this thesis. The first project explored solvent use in Winnipeg, and the second project investigated HLA-B*35 allele subtypes in HIV+ patients from Manitoba.

The solvent use study sought to investigate the relationship between solvent use and HIV in Winnipeg, Manitoba through an interdisciplinary multi-phase approach that integrated both community based research (CBR) and basic science research. Two distinct long-term research directions provided the rationale framework for this study. These state that HIV risk, in the context of solvent use, is affected by biological as well as social factors. To address both research directions, the intention was that phase one would be a community-based-research focused research study which would inform phase two, a basic science research study. We aimed to establish a research partnership with Sunshine House and to characterize what solvent use is and who solvent users are in the context of the Winnipeg solvent-using population through focus groups with solvent users and individual interviews with solvent users and key informants

From interviews with solvent users and key informants we learned that individuals who use solvents experience many disparities within the social determinants of health framework, outlined by the Public Health Agency of Canada, which address vulnerabilities and resiliency of Aboriginal peoples in Canada regarding HIV/AIDS. We have demonstrated that there is support within the community to work with solvent users and study solvent use. This early phase of research has provided a foundation to build a second phase of research involving the collection of biomedical samples to thoroughly investigate the impact of solvent use on the immune system and HIV disease. Furthermore, solvent use is certainly not unique to Winnipeg. Establishing a research approach in Winnipeg will be helpful in exploring the link between HIV and solvent use locally and internationally.

The HLA project investigated genetic links to HIV disease progression. The increased prevalence of HLA-B*35 within Aboriginal patients in Manitoba prompted an enquiry into the distribution of specific HLA-B*35 allele subtypes, at the high resolution level, in Manitoba. The hypothesis for this project was that the HLA-B*35 patient population enrolled in the Manitoba HIV Program will be enriched with HLA B*35 allele subtypes that associate with rapid progression. We aimed to genotype existing DNA samples using sequence-based methods and to complete a subset analysis (n=5) correlating high resolution HLA typing results with clinical data regarding disease progression ($CD4^+$ T cell counts and viral load over time, opportunistic infections and ethnicity.

The HLA-B*35 study aimed to identify the diversity and frequency of HLA-B*35 allele subtypes, at the high-resolution level, in the HIV+ population in Manitoba that presented to care between 2007 and 2010. We observed that 11 distinct HLA-B*35 allele subtypes exist within the population and that the most common allele subtypes are B*35:01:01G, B*35:03:01G, and B*35:08:01. Case studies reflected the overrepresentation of Aboriginal people infected with HIV in Manitoba, and the pressing issues of either late presentation to care or rapid disease progression within those who are HLA B*35. When considering both the allele subtype distribution data and the case reports, it is clear that allele subtypes associated with HIV rapid disease progression are enriched within this cohort and that these alleles may in fact be exerting an influence. This study lends support to the idea that HLA genotyping HIV positive patients, at the high resolution level, is a useful tool when trying to determine if a patient should be placed on antiretroviral therapy.

The interdisciplinary research approach used can be considered an experiment in new approaches to tackling problems at the intersection between complex biological and social issues.

Dedication

This thesis is dedicated to Margaret Ormond. Although terms like mentor and friend come to mind, they don't do our relationship justice. Thank you for waking me up.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1: HIV Burden of Disease

The HIV/AIDS pandemic has had an incredible global impact. As of 2011, there are an estimated 34 million people living with HIV worldwide. UNAIDS estimates that 2.5 million people were newly infected with HIV, and 1.7 million people died of AIDS related deaths in 2011 (1). In Canada, the number of people living with HIV/AIDS rose 11.4% from 2008 to 2011, which reflects new infections as well as treatments that improve the survival of people living with HIV (2).

Aboriginal people in Canada comprise a unique, overrepresented segment of the epidemic. Aboriginal peoples make up 3.8% of the general Canadian population and yet represent 12.2% of new infections, which is 3.5 times the incident rate compared to the general population. It is important to keep in mind that the term "Aboriginal" collectively represents First Nations peoples, Métis peoples, and Inuit peoples (2). Data from 2006 regarding Aboriginal AIDS cases showed that First Nations peoples are overrepresented within the Aboriginal subgroup, with 73.1% of AIDS cases compared to the Métis peoples (7.3%) and Inuit peoples (3.6%) (3).

The number of new infections in different exposure categories in 2011 was very different between Aboriginal Canadians and the general population, and also varied regionally. The burden of new infections in the general Canadian population was as

follows: men who have sex with men (MSM) (46.6%), heterosexual individuals from non-endemic regions (20.3%), intravenous drug users (IDU) (13.7%), and heterosexual individuals from endemic regions (16.9%). In contrast, within the general Aboriginal population, IDU account for 58.1% of new infections, heterosexual individuals account for 30.2% of the new infections, and MSM account for 8.5% of new infections. Additionally, Aboriginal individuals who are both IDU and MSM account for 3.1% of new infections (2).

While the HIV incidence rate in Canada appears to have stabilized since 2002, Manitoba and Saskatchewan have experienced continued increases in new cases. Saskatchewan has an incidence rate of 19.1 per 100,000, which is double the national average of 8.2 per 100,000, and Manitoba has an incidence rate of 12.3 per 100, 000. Regarding exposure categories, Saskatchewan's elevated rate is driven by intravenous drug use, and Manitoba's rate is driven by unprotected heterosexual sex (1, 4).

The Manitoba HIV Program has identified two key Manitoba issues. First, Aboriginal peoples are overrepresented in new cases to care (2, 4). Aboriginal peoples made up 15% of Manitoba's population and represented 53% of new cases to care in 2011, which was up from 43% of new cases in 2009 (2, 5). Within the Aboriginal new cases to care in 2011, heterosexual sexual transmission accounted for 71% of new cases, and IDU and MSM accounted for 19% and 10% of new cases, respectively (4).

The second key issue identified by the Manitoba HIV Program is late presentation to care. A "late presenter" is an individual who presents to care with <350 $CD4^+$ cells/mm^3 and "advanced HIV disease" is characterized by presentation to care with <200 $CD4^+$ cells/mm^3. In 2011, 64% of new cases qualified as late presenters, and 45% of these cases were Aboriginal individuals. Additionally, 38% of new cases in 2011 qualified as presenting to care with advanced HIV disease (4).

1.2: HIV virology

1.2.1: HIV Virion Structure

HIV virions are enclosed by a lipid bilayer, which originates from the host cell membrane, and includes exposed surface glycoproteins (gp120) and transmembrane proteins (gp41). Directly beneath the lipid bilayer is the matrix shell, composed of matrix proteins (p17). A conical capsid core, made of capsid proteins (p24), exists within the matrix shell and contains two copies of viral genome stabilized by nucleocapsid proteins (p7). The capsid core also contains three virally encoded enzymes called protease, reverse transcriptase, and integrase. Mature virions also contain the accessory proteins nef, vif, and vpr. Additionally, rev tat and vpu are accessory proteins that are not packaged by the virus (6).

1.2.2: HIV Transmission

HIV can be transmitted and acquired by the host at the mucosal surfaces of the male and female genital, anal, and oral tracts during unprotected sexual encounters. HIV can

also be transmitted orally from mother to child via breast milk. Non-mucosal avenues of transmission include the direct blood to blood contact that can occur during intravenous drug use and also mother to child transmission in utero (7). The plasma viral load has been shown to be associated with transmission risk (8).

1.2.3: HIV Life Cycle

Once HIV has entered the body and is brought into contact with target cells, the gp120 surface protein of HIV recognizes and binds to CD4 receptors located on host cells. This induces a conformational change and allows the virus to bind to a chemokine coreceptor. The CCR5 coreceptor binds R5 tropic viruses and is largely responsible for mucosal transmission. The CXCR4 coreceptor binds X4 tropic viruses and is associated with later stage disease. The binding between gp120, CD4, and a chemokine receptor induces a conformational change that exposes viral gp41 and subsequent fusion of the virion and target cell membrane. The viral core is then released into the target cell cytoplasm (9).

Once inside the host cell, viral uncoating occurs and the reverse transcriptase complex is generated (9). The original viral positive-sense single-stranded RNA genome is transcribed into viral complementary DNA (cDNA) (6) and then transported through the nuclear pore into the nucleus. Within the nucleus, the viral integrase enzyme integrates the cDNA into the host chromosome. The established HIV provirus can then enter latency or become transcriptionally active (9). The viral long terminal repeat segment

binds with both cellular and viral proteins to accomplish the transcription of proviral DNA into viral mRNA. The necessary cellular proteins are abundant in activated T cells and macrophages, and therefore, viral replication is more efficient in activated cells (10). Following transcription and translation, viral components are assembled at the host plasma membrane and mature virions are released via budding (9).

1.3: The Host Immune System

The human immune system can be divided into the innate and adaptive systems. The innate immune system is the first line of defense against pathogens and includes structures such as the epithelial and mucosal surfaces of the body, phagocytic cells (neutrophils, monocytes, macrophages), natural killer (NK) cells, and molecular mediators including complement proteins and chemokines. The innate immune system is non-specific and is not able to generate a memory response (11).

If a pathogen evades the innate immune system, it will encounter the adaptive immune system. The adaptive immune system is capable of generating an immune response to specific pathogens due to receptor diversity, and also possesses immunological memory. That is, if the host encounters a pathogen that has previously been encountered, a rapid and specific immune response can be generated. The adaptive immune system has two arms. The first arm is humoral immunity, which involves the generation of antibodies by B cells, and is effective against extracellular pathogens such as extracellular bacteria and parasites. The second arm is termed cell-mediated

immunity and primarily consists of $CD4^+$ T cells and $CD8^+$ T cells. $CD4^+$T cells are called T helper cells because they help B cells to generate antibodies. The $CD8^+$ T cells are called cytotoxic T lymphocytes (CTLs) because of their ability to destroy cells infected with intracellular pathogens, such as viruses and intracellular bacteria (11).

1.4: HIV Disease Progression

HIV is capable of effectively evading both the innate and adaptive immune systems to establish infection (8). HIV disease progression is split into three phases, summarized in **Figure 1.1**, and includes the short (weeks) acute phase, the long (years) chronic phase, and the final AIDS phase. In early acute phase, a failure of the innate immune system to control viral replication, combined with the absence of pre-existing HIV-specific humoral and cell mediated immunity, leads to a peak in plasma viral load and is associated with a high risk of transmission. This early phase also involves a steep decline in $CD4^+$ T lymphocytes (8) which is reflected more dramatically in the gastrointestinal associated lymphoid tissue (GALT) than in peripheral blood (12). It is now widely accepted that the early and continued destruction of GALT results in microbial translocation induced immune activation throughout the course of disease (13). Towards the end of the acute phase, the adaptive immune system responds with HIV-specific antibodies and CTLs, which result in an incomplete rebound of $CD4^+$T cells and a drop in viremia to the viral set point. The chronic phase of HIV infection is asymptomatic, yet progressive, and involves a chronic state of immune activation (8). Eventually, the ongoing destruction of

lymphoid tissue combined with immune activation driven immune exhaustion leaves the host's immune system unable to control AIDS defining opportunistic infections (14).

There are three main disease progression categories. The first is long term non progressors, and includes elite controllers, viremic controllers and viremic non-controllers. Long term non progressors share the characteristic of asymptomatic HIV infection over 10 years following seroconversion. The second main disease progression category is chronic progressors, who have symptomatic infection or are initiated onto anti-retrovirals (ARVs) within ten years following seroconversion. The last category of disease progression is rapid progressors. Individuals who are considered rapid progressors generally exhibit more than two $CD4^{+}T$ cell measurements below $350mm^{3}$ within three years following seroconversion, or are placed on ARVs within three years following seroconversion, or have AIDS defining disease or death within three years (15).

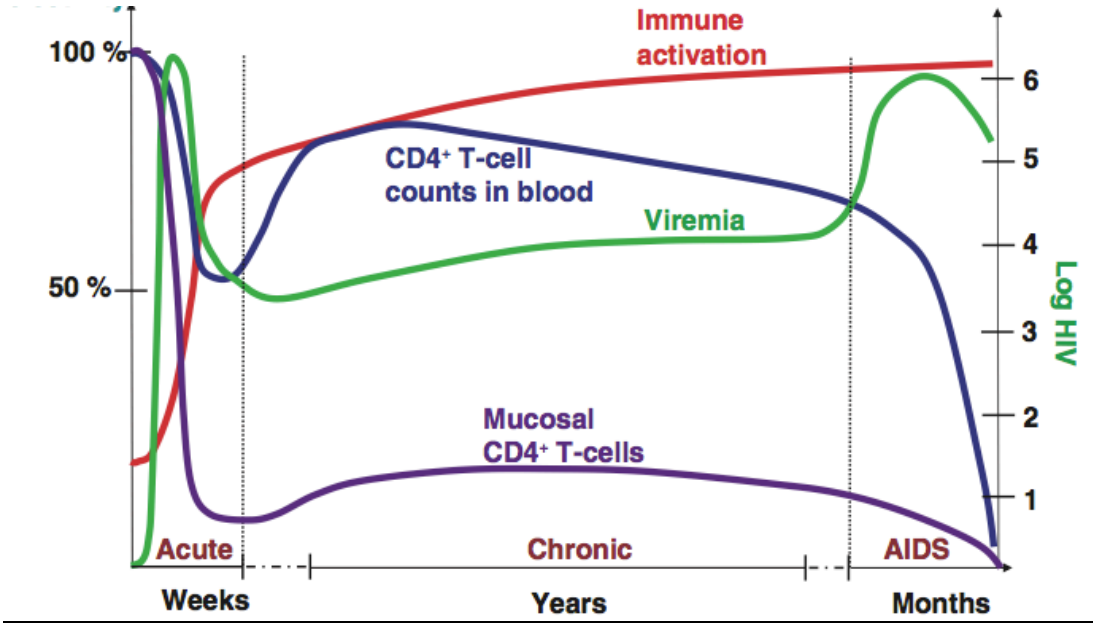

Figure 1.1: Typical HIV disease progression in the absence of antiretroviral therapy (Figure originally published by Grossman et al (16) and used with permission from Dr. Zvi Grossman)

The outcome of HIV disease depends on a complex interplay between both viral and host factors (17). This thesis presents two distinct projects, each of which explores a host factor present in the Manitoba HIV population that is relevant to Aboriginal populations. The first project uses a community-based research (CBR) approach and investigates solvent use, which is an environmental host factor. The second project investigates HLA-B*35, which is a host genetic factor, using a basic science approach.

1.5: HIV Environmental Risk Factor: Solvent Use

1.5.1 Solvents

Solvents belong to a large group of compounds known as inhalants, which are volatile at room temperature and produce an altered state of consciousness when inhaled. There are three major groups of inhalants and each group consists of a diverse collection of compounds. The first group is compounds that contain nitrous oxide, such as aerosol cans and anesthetics. The second group is volatile alkyl nitrites, which are often called "poppers". The third group, which are the subject of this thesis, is solvents (18).

Solvents are loosely defined as a broad group of readily available household substances including gasoline, correction fluid, lacquers, and glues. The routes of administration include sniffing, snorting, huffing, bagging, and spraying the compound directly into the oral cavity. Solvents are lipid soluble, and after being absorbed by the lungs they are quickly taken up by the central nervous system resulting in psychoactive effects (19).

1.5.2: Solvent Use

Reports from all around the world, including Mexico (20), Egypt (21), Colombia (22), Australia (23), India (24), South America (25), and the United States of America (26) have implicated solvent use as substance use habit among youth, primarily of low economic status. In Canada, adults have also been reported to use solvents (27).

The first reported example of inhalant use in Canada occurred in 1964 when a pharmacist in Winnipeg noted that young people were stealing and inhaling nail polish remover (28). Solvent use has been reported as a major issue facing Canada's Aboriginal population (29). The Winnipeg Street Health Report from 2011 reported that 6.3% of respondents identified using solvents regularly in the past year, which was three times higher than a Toronto Street Health Report from 2007. The Winnipeg report noted that there is a lot of stigma associated with solvent use and predicted that the actual number of solvent users is higher than what was reported (30).

1.5.3: Solvent Use and HIV

In 2010, a local epidemiological study investigated socio-demographic variables, drug-related risk factors and risk of blood borne pathogens within a Winnipeg Aboriginal intravenous drug using (IDU) population that also use solvents. Compared to Aboriginal IDU who did not use solvents, solvent users were found to be statistically younger, more likely to be infected with Hepatitis C virus (HCV), and more likely to have shared needles within the previous six months. The study found a trend between solvent use and HIV

prevalence with solvent users twice as likely to have acquired HIV than non-solvent users (17.5% vs. 8.3% respectively). Although the relationship was only a statistical trend, the study suggested more detailed studies of solvent use populations, including those that are non-IDU, are needed (29).

The hypothesized link between solvent use and HIV is largely supported by observations from individuals who work closely with the population and anecdotal evidence from local health practitioners. Regarding evidence from the community, Sunshine House, a community-based organization in Winnipeg that provides services to street involved individuals at risk for HIV and other blood borne infections, has estimated a self-reported prevalence rate of 20% amongst clients [Personal communication, Margaret Ormond]. The client base is largely homeless individuals, with a large proportion of Aboriginal peoples (80%) (31) and most clients are impacted either directly or indirectly by chronic solvent use. Regarding clinical evidence, staff from the Manitoba HIV Program, which is the primary organization for providing specialized HIV care in Manitoba, have noted rapid HIV disease progression among some solvent users [Personal communication, Dr. Ken Kasper].

1.5.4: Biological Implications of Solvent Use: Microbial Translocation

As mentioned above, the acute and chronic phase of HIV infection involve the extensive depletion of $CCR5^+$ $CD4^+$ T cells, which are normally abundant in mucosal tissue including the gut-associated lymphoid tissue (GALT) of the gastrointestinal (GI) tract. A

healthy gastrointestinal mucosa prevents microbial products from crossing the barrier between the GI tract and systemic circulation therefore the extensive depletion of GALT cells leads to a disruption of GI mucosal integrity. The compromised mucosal barrier allows for microbial products to translocate from the intestinal lumen into the systemic circulation (12, 13, 32). One such bacterial product, lipopolysaccharide (LPS) or endotoxin, is a cell wall component of gram negative bacteria and acts a potent activator of the immune system (13). The persistent exposure to LPS as well as other microbial products that activate the immune system can lead to a chronic state of immune activation that characterizes the second phase of HIV disease (33).

Immune activation can both benefit and harm the host. On one hand it readies the host to battle against a pathogen and involves polyclonal B cell activation, increased activation and turnover of T cells, and also increases the amount of pro-inflammatory cytokines and chemokines. On the other hand, chronic immune activation can lead to strain on T cell homeostasis, clonal exhaustion, and also reduce memory T cell pools (34). Persistent immune activation also generates activated $CD4^+$ T cells, the target cells of HIV (34). Indeed, immune activation has been demonstrated to be a more accurate predictor of disease progression than $CD4^+$ T cell count (13) or plasma viral load (32).

A growing body of evidence supports the link between microbial translocation associated immune activation and HIV disease severity and progression. For example, HIV-1 infected individuals with AIDS have been demonstrated to have higher plasma LPS

levels than uninfected individuals (13, 35), and elevated plasma LPS levels have been demonstrated in advanced HIV-1 and HIV-2 infections and correlates with severity of disease (35).

Clinical staff from the Health Science Centre in Winnipeg have performed tracheal and GI endoscopies on solvent users and observed an abundance of erythema and inflammation of the upper GI tract. Staff from the Manitoba HIV Program have also noted rapid HIV disease progression among some solvent users [Personal communication, Dr. Ken Kasper]. The relevance of this in terms of HIV disease risk or progression is unknown.

1.5.5: Biological Implications of Solvent Use – Natural Killer Cells

Nitrite inhalants are a separate category of inhalants from solvents, and have been linked to HIV previously, although whether they influence susceptibility or disease progression remains inconclusive. Murine models showed that nitrite inhalation impaired $CD4^+$ T cell-dependent B cell activity, inhibited $CD8^+$ CTL induction, and impaired macrophage tumoricidal activity. Interestingly, NK cell cytoxicity was also found to be impaired (36). Natural killer cells bridge the innate and adaptive immune system for combating viral infections, and are able to kill HIV infected cells (37). Additionally, elevated levels of pro-inflammatory cytokines such as TNF-α (tumor necrosis factor alpha) were observed (36) which is important because increased TNF-α promotes HIV viral replication. A human study from 1991 showed that nitrite inhalation

was immunosuppressive and reduced absolute lymphocyte counts while impacting the NK subset most dramatically. While lymphocyte counts were found to rebound following drug cessation, the NK subset required additional time to rebound (38). Increased exposure to nitrite inhalants was found to be associated with more dramatic effects in both the murine and human models. Whether or not solvent use has a similar immunosuppressive role is unknown.

The following sections introduce the second project presented in this thesis, which explores HLA-B*35 allele subtype frequencies in HIV infected patients from Manitoba.

1.6: HIV Genetic Risk Factor: HLA-B*35

1.6.1: Human HLA

The major histocompatibility complex (MHC) genes are vertebrate genes responsible for self vs. non-self recognition and are clinically important regarding organ transplantation and associations with disease (39). In humans the MHC region is a ~4Mb (40) region on chromosome 6p21.3 and referred to as the human leukocyte antigen (HLA) region (39). The HLA region codes for a variety of polymorphic genes including the class I and II HLA molecules, which are important for pathogen-peptide presentation to the immune system (41). The HLA genes are the most diverse in the human genome and their diversity is concentrated to specific regions that code for peptide binding groove of HLA class I and II cell surface receptors (42).

HLA receptors are essential for the presentation of antigens to T lymphocytes. Class II HLA loci, including HLA-DR, HLA-DQ, and HLA-DP, code for receptors on the surface of antigen presenting cells which bind extracellular peptides and present peptides to $CD4^+$ T cells. Class I HLA loci, on the other hand, code for receptors that are present on the surface of virtually all cells that are nucleated. Class I HLA are responsible for presenting intracellular peptides to $CD8^{+-}$ T cells (42).

1.6.2: Human HLA and HIV

An infection with HIV results in an immune response against viral epitopes presented to $CD8^+$ T cells by HLA Class I. HLA gene products play important roles at several stages of HIV-1 infection. They select the repertoire of HIV epitopes presented to $CD4^+$ and $CD8^+$ T lymphocytes, are involved in induction of NK cell response, and exert pressure on the virus that leads to viral mutations and escape (41). The specific HLA genes utilized to present antigen can also be used to make predictions about disease outcome (2). The earliest $CD8^+$ T cell responses appear to be critically important in controlling viremia and establishing a viral set point. This early response is narrower in specificity than later responses and has been associated with lower plasma viremia for the following 2-3 years in the absence of therapy (43).

HIV can escape CTL response via viral escape mutations. Escape mutations can result in the modification of either the ability of HLA receptor to bind a viral peptide or the ability of the CTL to recognize the HLA-peptide complex. Viral mutations that evade the host

immune response without abrogating viral viability allow the virus to continue replicating. On the other hand, viral mutations that occur in regions that are essential to viral function reduce viral viability (44).

1.6.3: HLA Typing

With excess of 50 genes and 8000 alleles, the MHC is amongst the most complex and polymorphic regions within the human genome (45). Low resolution HLA typing was historically done through antibody-based serological testing, but higher resolution typing is now accomplished by modern molecular methods including sequence-specific-primer (SSP) typing, sequence specific oligonucleotide (SSO) probe based typing, and DNA-sequence based methods (46). Molecular methods have proved useful for both clinical and research applications (47). For example, routine HLA typing of all HIV positive individuals is conducted in Canada to screen for Abacavir hypersensivity (5) which occurs in individuals who possess the HLA-B*57:01 allele (48).

HLA typing resolution refers to the level of ambiguity when determining the exact alleles present in an individual. Low resolution typically refers to two-digit typing, and this set of digits represents allele type and typically matches historical serological antigen grouping (ex: HLA-B*35). High resolution typing includes the first set of digits as well as up to three additional sets of numbers. The second set of digits (ex: HLA-B*35:01) designates the allele subtype and is numbered based on order of discovery. The third set of digits (ex: HLA-B*35:01:01) distinguishes alleles that have synonymous

substitutions that do not change the amino acid sequence or protein, and the last set of digits (ex:HLA-B*35:01:01:01) distinguishes alleles that possess sequence differences in non-coding regions (46).

1.6.4: HLA and HIV Disease Progression

Several HLA-B genes have been associated with rapid and slow HIV disease progression. Genes associated with slow progression include HLA-B*27 and HLA-B*57, which have been shown to be elevated in several cohorts enriched with slow-progressors (44, 49). Both HLA-B*27 and BHLA-B*57 have been shown to tolerate mutations in their epitopes (44). On the other hand, HLA-B*35 and HLA-B*53 have been shown to be associated with rapid CD4 decline and therefore rapid disease progression (50). An individual that is homozygous for a specific gene locus will have two of the same alleles at that locus, whereas an individual that is heterozygous, will have two different alleles (51). Homozygocity of MHC Class I alleles has also been associated with rapid disease progression (52) because a homozygous individual will display a more limited selection of class I receptors on their cell surface and therefore have less diverse antigen presentation to CTLs than a heterozygote (53). Heterozygotes can present a wider variety of viral peptides and therefore mount a more productive immune response (53). Recognizing which HIV positive patients have a genetic predisposition for accelerated disease progression can be useful for physicians when deciding when a patient should be placed on anti-retroviral medications (ARVs) (54).

1.6.5: HLA-B*35

The HLA-B*35 gene is universally distributed and also one of the most common genes (55). The frequency of the allele varies between populations. For example, blood donor data records from around the world show that 31.5% of blood donors in Costa Rica have at least one HLA-B*35 allele, whereas in China, the frequency is 6%. The frequency also fluctuates between ethnic groups of the same population. American blood donor records show that 20.9% of American Caucasians from Bethesda have HLA-B*35 whereas the allele is present in 11.7% of African American's from the same geographic region (56).

To date, 241 unique HLA-B*35 alleles have been identified by various HLA typing methods (45), making it one of the most polymorphic HLA gene (55). Diversity of HLA-B*35 at the allele subtype level has also been demonstrated to exist between areas of the world. For example, a Croatian study identified four HLA-B*35 allele subtypes within their cohort (55), and an Italian study found the presence of thirteen allele subtypes (57). Polymorphisms in HLA-B*35 have been associated with various conditions including both chronic Hepatitis B Virus and Hepatitis C virus infection (58), *Chlamydia pneumoniae* (59), and hantavirus (60).

1.6.6: HLA-B*35 and HIV

The relationship between HLA-B*35 and HIV disease progression was established in the late 1980s and 1990s by studies that demonstrated that HLA-B*35 individuals were

more likely to progress quickly to AIDS (61-63). HLA-B*35 was also identified as a risk factor for illnesses that define AIDS (62). HLA-B*35 has continued to be associated with rapid disease progression (44, 53, 54) and homozygocity for HLA-B*35 has been shown to be associated with worse outcome (54).

HLA-B*35 can be divided into two subtype groups, PX and PY, reflecting amino acid differences in the peptide binding groove. The PX subtype group (35:02, 35:03, 35:04 and the related 53:01) has a proline at position two, does not have specific preference at position 9 (hence PX), and has been demonstrated to be associated with HIV rapid progression. The PY group (35:01 and 35:08) also has a proline at position 2, but has a tyrosine at position 9 (hence PY), and has been demonstrated to have no effect on disease progression (50, 54, 64, 65). This difference in response appears to be attributed to differences in $CD8^+$T cell responses that are restricted to specific PX and PY epitopes although the exact mechanism is unknown (64). It has also been demonstrated that homozygosity for HLA-B*35, regardless of PX or PY distinction, is associated with rapid disease progression (54).

1.6.7: HLA in Manitoba

A recent study from the Manitoba HIV Program looked into the relationship between HLA type, $CD4^+$ T cell decline, and opportunistic infections in 102 new patients enrolled into care in 2010. It was shown that there are high rates of HLA-B*35 and HLA-B*51 alleles, which have previously been associated with rapid $CD4^+$ T cell decline, and that

HLA-B*35 was more likely to be found in Aboriginal patients. The protective allele, HLA-B*5701 was absent from the Aboriginal population and present only in Caucasian patients. Of the 15 patients that were homozygous for their HLA type, 60% were Aboriginal (5).

The allele subtype frequency of HLA-B*35 in Manitoba is unknown. Since HLA-B*35 can be divided into two distinct allele subtype groups with different disease progression patterns, it is important to determine which subtypes associated with rapid progression are present, and at what frequencies, within the Manitoba cohort. This project utilized high resolution HLA genotyping methods to determine HLA-B*35 subtype frequencies in HIV positive individuals from Manitoba.

1.7: Social Determinants of Health

The social determinants of health are a reflection of a population's living conditions and affect both lifespan and quality of life (66). The major social determinants of health associated with health disparities in Canada are Aboriginal status, income, and place (67). The Canadian government has been criticized by academic scholars (66-69) for failing to develop effective public policy addressing health disparities.

Researchers have suggested that the prevalence of HIV in Canada's Aboriginal population has been influenced by the effects of discrimination, cultural disruption, and systemic poverty (70). The Public Health Agency of Canada (PHAC) has outlined ten

categories which address both the vulnerability and resiliency of Aboriginal peoples in Canada regarding HIV/AIDS. These social determinants of health are culture, social environment and support, income/education/employment, physical environments (including geographic isolation, housing, homelessness, and prisons), personal health practices/coping skills (including sexual behaviors, drug use, sex work and HIV testing), healthy child development, biology and genetic endowment, health services, gender, and finally, the impact of the residential school system (3).

The components of this thesis aim to incorporate a thoughtful consideration of the social determinants of health influencing the prevalence of HIV in Aboriginal peoples in Manitoba.

1.8: Gaps in Knowledge

The HIV and solvent use component of this thesis addresses several major gaps in knowledge regarding solvent use and solvent users in Manitoba and builds the foundation for further biomedical inquiry into the impact of solvent use on the immune system and HIV disease. There has never been a comprehensive study focused primarily on solvent users in Manitoba. Manitoba seems to have a unique population of adult solvent users that do not fit the criteria and characteristics of a typical solvent user defined in the literature. The majority of the literature available is dated, largely from a non-Manitoban perspective, and focused on youth.

The HIV and HLA-B*35 component of this thesis addresses the gap in knowledge that HLA-B*35 subtype frequencies in Manitoba are not known.

1.9: Central Aim of Thesis

The Manitoba HIV Program has identified two key Manitoba issues. First, Aboriginal peoples are overrepresented in new cases to care (4). The second key issue identified by the Manitoba HIV Program is late presentation to care. The Manitoba HIV Program 2011 Program Update Report concluded with a discussion of factors influencing the over-representation of Aboriginal peoples in the Manitoba HIV epidemic and stated that an effective response called for the "comprehensive understanding of contextual factors and must involve multiple contributing voices" (4). Taking into account that both biological and social factors influence the dynamics of HIV disease, the components of this thesis aim to employ an interdisciplinary approach to exploring host factors which may have important consequences for HIV risk and disease progression in Manitoba and be relevant to Aboriginal populations.

1.10: Project One - Solvent Use Project

1.10.1: Rationale

The hypothesized link between solvent use and HIV is largely supported by observations from individuals who work closely with the population and anecdotal evidence from local health practitioners. Two distinct long-term research directions provided the

rationale framework for this study. These state that HIV risk, in the context of solvent use, is affected by biological as well as social factors.

Regarding the biological rationale, the research team hypothesized that solvent use damages mucosal surfaces and leads to increased microbial translocation and subsequently higher levels of immune activation. Additionally, it was hypothesized that solvent use impacts the natural killer subset of cells. Both of these mechanisms may have important consequences for HIV risk. Regarding the social rationale, the research team hypothesized that solvent users experience social inequalities, such as discrimination and exclusion, which result in many unmet needs. Additionally, it was hypothesized that solvent users may be involved in risky behaviors that may increase HIV risk.

This project aimed to investigate the link between solvent use and HIV through a multi-phase interdisciplinary research project. To address both research directions, the intention was that phase one would be a community-based-research focused research study, which would inform phase two, a basic science research study.

For phase one of the project, a set of clear short-term objectives and major research questions were established.

1.10.2: Objectives

1. Establish a research partnership with Sunshine House
2. Characterize what solvent use is and who solvent users are in the context of the Winnipeg solvent-using population through focus groups with solvent users and individual interviews with solvent users and key informants

1.10.3: Major Research Questions

1. What is solvent use in the context of Winnipeg, Manitoba?
2. Who are solvent users in the context of Winnipeg, Manitoba?
3. To what extent is the research team and approach accepted by the solvent-using population?
4. To what extent is a second phase of project, involving biomedical research, feasible?

1.11: Project Two: HLA B*35 Project

1.11.1: Rationale

An epidemiological study, from Mackenzie et al, of all patients with HIV new to care in the Manitoba HIV Program in 2010 showed that individuals of Aboriginal ethnicity were overrepresented and presenting to care with significantly higher disease burden than Caucasian patients. The study also showed that nearly one quarter of the Aboriginal patients possessed at least one HLA B*35 allele, compared to approximately 14% of Caucasian patients. Additionally, there were low rates of protective alleles (HLA B*27

and B*57) and high rates of homozygocity, which are associated with rapid disease progression, within the Aboriginal cohort (5). The allele subtype frequency of HLA-B*35 in Manitoba is unknown. Since HLA-B*35 can be divided into two distinct allele subtype groups with different disease progression patterns, it is important to determine which subtypes associated with rapid progression are present, and at what frequencies, within the Manitoba cohort.

1.11.2: Hypothesis

The HLA-B*35 patient population enrolled in the Manitoba HIV Program will be enriched with HLA B*35 allele subtypes that associate with rapid progression.

1.11.3: Objectives

1. Genotype existing DNA samples using sequence based methods
2. Complete a subset analysis (n=5) correlating high resolution HLA typing results with clinical data regarding disease progression ($CD4^+$ T cell counts and viral load over time, opportunistic infections) and ethnicity

Chapter 2: Materials and Methods

2.1: Solvent use and HIV project

2.1.1: Introduction

This project aimed to investigate the link between solvent use and HIV through a multi-phase interdisciplinary research project. The research project was split into two phases. The first phase was a qualitative community-based-research (CBR) focused research project which aimed to inform the second biomedical focused phase of research. Chapter 3 of this thesis represents work done in the first phase.

2.1.2: Research Team

The research team consisted of a graduate student, Courtney Bell, from the department of Medical Microbiology, the graduate student's supervisor, Dr. Keith Fowke, (herein referred to as the PI), a Sunshine House representative, Margaret Ormond (herein referred to as the community-PI), a local epidemiologist, Dr. John Wylie, and a social scientist, Dr. Javier Mignone. Dr. John Wylie's research associate, Chelsea Jalloh (herein referred to as the RA),was also involved in the analysis phase of the research project.

2.1.3: Research Site: Sunshine House

This study was done in partnership with Sunshine House, a not-for-profit community-based organization that provides services to street-involved populations in downtown, Winnipeg. The mission of the organization is "to address determinants of health for the benefit of the neighborhood and poly drug users who are entirely or mainly homeless"

(31). Sunshine House has successfully engaged vulnerable populations for over a decade and has provided services such as clothing, laundry, bath, clean needles, safe crack kits, condoms, and food. Operating with a harm reduction approach and the principle of inclusion, Sunshine House does not have a requirement that clients be sober to receive services. Sunshine House has always been inclusive to solvent users.

2.1.4: Interdisciplinary Research Approach

There were several motivating forces from different disciplines informing the development of the study:

i) Clinical evidence from the Manitoba HIV program included the observation of rapid HIV disease progression and upper gastrointestinal mucosal inflammation in HIV positive patients that were solvent users.

ii) ii) Local epidemiological data showed that Aboriginal injection drug users (IDU) that also used solvents had elevated rates of HIV, Hepatitis C and needle sharing compared to non-solvent using IDU (29).

iii) Support staff at Sunshine House observed the presence of high rates of self-reported HIV status in their solvent-using clientele (31).

The study sought to explore the relationship between HIV and solvent use in Winnipeg, Manitoba using an interdisciplinary approach. The National Institutes of Health (NIH) Office of Behavioral and Social Sciences Research published a paper in 2008 that outlined the organization's vision of interdisciplinarity as a method to improve public

health. They identified interdisciplinarity as “a more robust approach to scientific integration in the sense that team members not only combine or juxtapose concepts and methods drawn from their own different fields, but also work more intensively to integrate their divergent perspectives, even while remaining anchored to their own respective field” (71). Additionally, Newell (72) states that an interdisciplinary approach is justified when the system studied is complex. Interdisciplinary studies have also been defined as “what happens when researchers go beyond establishing a common meeting place to develop new method and theory crafted to transcend the disciplines in order to solve a problem” (73).

The research team included members with backgrounds in basic science, epidemiology, social science, and community-based research, and identified that the relationship between solvent use and HIV constitutes a complex system when approached from either a basic science, clinical, epidemiological or community perspective. Multiple disciplines have proposed a link between solvent use and HIV, but little has been done to explore potential mechanisms contributing to the observed phenomena. The team believed that a contributing factor for the slow progress on this important topic was related to the researchers remaining limited in their respective disciplines. Thus, an interdisciplinary approach was chosen that integrated basic science and CBR.

Newell (72) suggests that those undertaking interdisciplinary research “need to learn something new about a discipline each time they make use of it.” Newell argues that

researchers committed to an interdisciplinary approach are not required to be experts in the disciplines used, but rather they should have a general understanding and embrace the portions that are relevant to their research. At the onset of the study, the graduate student involved possessed a firm background of basic science knowledge from the fields of microbiology, genetics, and biochemistry. However, the student had little exposure to social sciences and community-based research. Borrego and Newswander (74) state that "graduate students and their training programs are recognized as central to increasing interdisciplinary research capacity," and follow up this statement by pointing out that the literature available regarding learning outcomes, methods, and benchmarks for graduate students undertaking interdisciplinary projects in science are virtually absent. The graduate student approached CBR with a learn-as-you-go method combined with the strong mentorship influence of the community PI. This method of learning was supplemented with the student's attendance to a Community-Based Research in Aboriginal Health summer institute, and the acceptance to a HIV-focused national fellowship program (University Without Walls) with a priority of training interdisciplinary researchers. Additionally, the research team's epidemiologist and social scientist were part of the graduate student's advisory committee and provided guidance throughout the project.

2.1.5: Community-Based Research Approach

Community-based approaches to research encourage researchers to engage with the participants and communities in meaningful ways (75). The research team approached

Margaret Ormond, the co-director of Sunshine House (at the time) to be involved in the development of the research priorities and design as the community principal investigator. Ms. Ormond has extensive background in community-based research, is an experienced research nurse, and has developed long-term and respectful relationships with Sunshine House clients. Under the guidance of Ms. Ormond, the graduate student was introduced to staff, clients, and Board members of Sunshine House. The student was also introduced to service staff at several other organizations that provide services to solvent users and street involved individuals in Winnipeg. Over the course of the project, the student's relationship with Sunshine House and involvement with other local solvent-use related research or interest groups developed further.

The community-based research approach was embraced at several stages within the research project and discussed below:

i) Prior to commencing the research, two informal dinners were held at Sunshine House and solvent-using clients were informed and invited by word of mouth. The graduate student and Ms. Ormond prepared the dinners. The basic science principal investigator, Keith Fowke, attended one of the dinners. The research team introduced the project to dinner guests and inquired about interest and feasibility of the approach. Dinner guests encouraged the research team to move forward.

ii) The project was introduced to the Board of Directors of Sunshine House and required their unanimous agreement to begin. The Board received updates on project progress throughout the project.

iii) The recruitment phase of the research study involved word-of-mouth sharing between potential participants

iv) The interview tools were modified following the first focus group to include subject matter that the research team had not originally planned on discussing.

v) During the interviews, participants were asked to give feedback on both the design of the current project, and advice on how later phases of the research, involving a biomedical focus, should be designed. Participants were also asked to discuss practical applications of the research as well as ideas regarding knowledge translation.

vi) Preliminary results were compiled, distilled, and discussed with a group of solvent users at an SOS (There is no consensus of what this acronym stands for. This is a group of solvent users that meets weekly at Mt. Carmel clinic) group meeting as a data verification step. The preliminary results were then presented at research conferences.

vii) The research team aimed to network with other local, national, and international researchers interested in solvent use research as well as local community organizations that provide services to solvent users. Two meetings were hosted by the research team. For the second meeting, two individuals who identified as solvent users were invited to be a part of discussions. The preliminary results

that had undergone data verification were presented at the second meeting as well.

viii) A second data verification meeting was held at the SOS group prior to submitting the final version of the thesis to Graduate Studies. Key points addressed in the discussion chapter of the thesis were discussed with the group.

2.1.6: Ethics

Ethics was approved for this project through the University of Manitoba Research ethics board. The study was called, "H2010:380 - Sniffing around the Issue: A preliminary investigation into the relationship between solvent use and HIV risk" and was approved January 13, 2011 (**Appendix B**). An annual approval was granted on October 31, 2012 (**Appendix B**)

Focus groups were hosted around a hot dinner, and once participants had settled into seats around the table, the community PI and graduate student gave a brief introduction to the project and then passed out informed consent documents (**Appendix C**). Participants read through the documents and the community PI and graduate student were present to answer inquiries. Voluntary informed consent was obtained from each participant. Individual interviews with solvent users were completed by the community PI, who obtained voluntary informed consent from each participant. Individual interviews with key informants were completed by the graduate

student, who obtained voluntary informed consent from each participant. All of the signed consent forms were compiled and locked in a secure cabinet.

2.1.7: Participants and Recruitment

The study used a purposeful sampling strategy (76). Potential participants (solvent users) for focus groups and individual interviews were approached during Sunshine House drop-in hours as well as offsite at known hang out places by the community PI. Potential participants were invited to participate and given a card with contact information and interview times were negotiated over the phone or in person.

Potential key informant participants were approached either in person or via email to participate in the study and interview times were negotiated. Key informants included four service providers, one physician, and one researcher.

All participants were compensated with $25 cash and two bus tickets.

2.1.8: Data Collection

Three focus groups were done at Sunshine House with the graduate student and community PI present. The first group consisted of five men, the second had three women, and the last focus group was a mixed group comprised of two men and one woman. Each focus group was done around a hot meal shared by group participants and the research team. Participants were first asked if they felt comfortable with the focus

group being recorded, and if there was consensus, the recorder was turned on. Next, the study was explained to participants and then participants were asked to provide voluntary informed consent. Once consent was provided, the community PI led the focus group question process and the graduate student also participated in asking questions and was involved in discussions. The graduate student took detailed notes during the focus group. Focus groups ran between an hour and 90 minutes in length. The main theme of the focus groups was "Concerns and Perspectives." (Tool attached **Appendix D**). Participants were asked to talk about their main concerns and if they had concerns regarding health and infectious disease. Participants were also asked to discuss the reactions they received from other people due to them using solvents. Lastly, participants were asked to discuss their ideas and suggestions regarding research. During the first focus group, participants expressed concern about the lack of treatment options for solvent users in Winnipeg. Questions about treatment were then built into the next focus groups, as well as individual interviews with both solvent users and key informants. At the end of each focus group, participants were asked to bring up topics that had not been discussed.

Individual interviews with solvent users were conducted by the community PI at Sunshine House. Each participant was asked if they felt comfortable with the interview being recorded before the recorder was turned on. Next, the study rationale was described, and voluntary informed consent was provided. Individual interviews were done with snacks present. Individual interviews with solvent users were done in one or

two sessions, which ran between thirty minutes to one hour in length. The theme for the interview tool (**Appendix E**) associated with the first session was "Personal History" and participants were asked to talk about their histories of using solvents, as well as patterns, habits and health concerns. The theme for the second set of questions was "Current experience and patterns of use/Social networks." Participants were asked to talk about their current usage and experiences with solvents, their social networks, their concerns, and ideas about research.

Individual interviews with key informants were conducted by the graduate student at various locations negotiated prior to the interview. Each key informant was asked if the interview could be recorded, provided with information about study rationale, and asked to provide voluntary informed consent. Key informant interviews ran between ten and thirty minutes in length. Key informants were asked (Interview tool in (**Appendix F**) to talk about their personal and professional connections to the solvent using population and how that relationship has evolved over time. Key informants were also asked to discuss their experience with diseases within the population and their cautions and advice regarding research.

Confidentiality and privacy were considered. Participants for each interview type were assigned a study code designation. Notes and transcripts were referenced by the study code and were not labeled with participant's names or occupations. All participants were provided with a $25 cash honorarium and two bus tickets as compensation for

their time. The interview tool for key informants included questions regarding their involvement and history with the solvent using population, their advice and concerns regarding research.

2.1.9: Data Analysis

All interviews were recorded. Transcriptions were done by the graduate student. Focus groups transcriptions were completed with the assistance of notes taken during the focus groups.

The analysis phase blended a phenomenological analysis approach with a grounded theory analysis approach, as outlined by Creswell (76). During the data collection phase, the focus group and interview data was analyzed for early emerging patterns. This practice allowed the research team to further refine the research questions and interview tools to reflect concerns of the population that had not been originally included as topics of discussion (77). First, transcripts were read several times and examined for important themes. This phase of coding, termed “open coding” by Creswell in his discussion on grounded theory, was done by three team members: the graduate student, the community PI, and the RA. A consensus was reached regarding a list of themes with preliminary subthemes for each set of interview. This triangulation approach relied on the convergence amongst the research team, which is a method of data verification (77).

The remainder of the analysis roughly followed a phenomenological analysis approach. For each theme identified, a document was created that included all of the transcript sections relevant for that particular theme. Themes that appeared in more than one interview type had separate documents created for each interview type. During this phase of coding, the graduate student, community PI and RA split the work evenly for interviews conducted with solvent users (focus groups and individual interviews), and the graduate student did the work pertaining to key informants. Once themed documents were created, the graduate student completed the remainder of the work with the guidance of team members. Each theme document was examined for previously identified and emergent subthemes and distilled into spreadsheets using Microsoft Excel. Within each interview type, a spreadsheet was created for each theme with participants on the vertical axis and subthemes across the horizontal axis (**Figure 2.1**). At this phase of coding, themes and subthemes were subject to reorganization. Quotes regarding specific subthemes were condensed into paraphrased notes and compelling quotes.

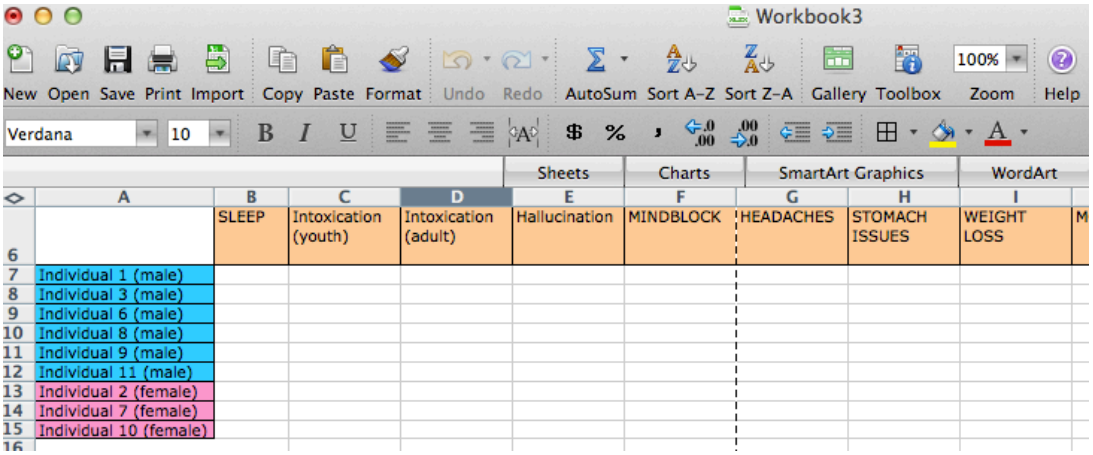

Figure 2.1: Representative spreadsheet layout used during analysis of qualitative data. This figure is part of the table used to analyze individual interview data for the theme of "Physical Effects". Interview participants are on the vertical axis, and subthemes are on the horizontal axis.

Once this phase of coding was complete, the graduate student met with Dr. Javier Mignone, the research team's social scientist, and discussed a write-up strategy. At this stage, the data was triangulated and assessed for patterns between interview types (77) and themes from each interview type were concentrated into a final over-arching list of themes and subthemes. This reorganization and shifting of themes and subthemes occurred throughout the writing and editing processes.

The data included a mix of subthemes that were specifically discussed during individual interviews, and subthemes that emerged without direct prompt. Responses from discrete questions were counted and written such that they were summarized into statements and the variation in responses was reflected. Topics that emerged without direct prompt were summarized into statements. Compelling quotes were added to statements to illustrate points and enrich the results. Differences between male and female points of view were included when present within the data generated from focus groups and interviews with solvent users. Gender differences were not considered with the key informant data. Finally, statements from the three data sets were compiled together into paragraphs and organized into themed sections.

2.1.10: Data Verification

Early in the data analysis process, the data was examined and distilled into key points for the purpose of presenting preliminary data at research conferences. The key points generated were discussed with a group at an SOS meeting at Mt. Carmel clinic. The SOS

group meets weekly and consists of individuals who identify as solvent users. With the assistance of the RA and Mt. Carmel SOS facilitators, key points were presented and discussed with SOS group attendees for approximately one hour to check the validity of the researcher's preliminary analysis.

A second data verification meeting was held with the SOS group prior to submitting the final version of the thesis to Graduate Studies. The discussion chapter was distilled to key points which were discussed with the group for approximately one hour.

2.2: HLA-B*35 Project

2.2.1: Cohort

This study involved HIV-patients enrolled into care by the Manitoba HIV Program (Health Science Centre HIV clinic and Nine Circles Clinic) between 2007 and 2010 who were identified as being heterozygous or homozygous for HLA-B*35. Clinical data was available for patients enrolled in 2010.

2.2.2: Ethics

A retrospective ethics certificate was obtained from the University of Manitoba Research Ethics Board for the samples obtained between 2007-2009. An ethics certificate was also obtained for the samples and case studies from 2010.

2.2.3: Samples

Between 2007 and 2010, whole blood samples (5-7mL EDTA sample per patient) were collected as part of routine clinical care from HIV+ patients in Manitoba. Samples were received by Canadian Blood Services (CBS) within seven days of collection. DNA was isolated and purified by CBS with Qiagen MagAttract DNA blood mini M48 Kits which is an automated purification technique that involves lysing original blood samples with specialized buffers. The DNA present in the sample binds to the silica surface of magnetic particles and can be obtained by washing the particles first with a series of buffers and lastly purified water. DNA was then diluted with distilled H_2O to a final concentration of 25 ng/μL. Low resolution B-locus HLA typing was done by CBS with One Lambda Lab Type SSO B Locus Kits. Low resolution B-locus HLA typing is done routinely by CBS to screen for HLA-B*57 because HLA-B*5701 is associated with hypersensitivy to Abacavir, an antiretroviral medication. DNA was cryopreserved and stored at CBS until collected and transferred to the University of Manitoba by the graduate student.

2.2.4: Sequence Specific Primer (SSP) Typing

Of the original 172 samples, a subset of the 47 most recent samples (collected between 2008 and 2010 from Manitoba) were typed using commercial HLA-B*35 sequence specific primer (SSP) kits (Table 4.1).

2.2.4.1: Reagents

10x Tris-Borate-EDTA (TBE) buffer: 108g Tris base, 55.0g Boric Acid, dissolved in 900mL deonized H_20. 20mL Na_2 ethylenediaminetetraacetic acid (Disodium ETDA) was added, and final volume was adjusted to 1L.

1x TBE buffer: 100mL 10x TBE buffer dissolved in 900mL deonized H_20.

Gel Electrophoresis buffer (1x TBE containing 0.5μg/mL Ethidium bromide buffer (EtBr): 500mL 1x TBE, 250μL Ethidium bromide (EtBr). Stored at 4°C.

The One Lambda Micro SSP HLA-B*35 DNA Typing kits are designed to discriminate between different HLA-B*35 allele subtypes using a method that employs both polymerase chain reaction (PCR) and gel electrophoresis. Each kit can test one patient sample and includes a 96-well format plate and a pre-mixed Micro SSP™ D-mix. The HLA-B*35 kit has 46 active wells, containing pre-optimized primers dried to the bottom of the wells, and one negative control reaction tube. The active wells also contain an internal control, which is from the human β-globuilin gene.

2.2.4.2: Plate Preparation

Kits were stored in a -70°C freezer until used and DNA samples were stored at -20°C until needed. Prior to beginning a kit, the Micro SSP™ D mix, primer set tray, and DNA sample to be used were removed from the freezers and placed in a biological safety

cabinet (BSC). The tray was placed in a 96 slot cooling rack. The DNA sample to be used was nano-dropped to determine if it was at the correct concentration and A260/A280 ranges prior to use. The kit directions required that DNA samples be at a concentration between 25-200ng/μl and have an A260/A280 ratio of 1.65-1.80.

Once the Micro SSP™ D mix was thawed and the DNA sample was determined to be acceptable, the plate preparation procedure began. The tray label was removed and the DNA sample was mixed thoroughly by vortexing for 5 seconds. One μL of distilled H_2O (The DNA diluent) was added to the negative control reaction tube (H1) of the kit.

Next, the master mix was prepared by adding reagents to the pre-made Micro SSP ™ D mix. Three μL (5 units/μL) Taq polymerase was added to the micro SSP™ D mix tube and vortexed for 5 seconds. Taq polymerase was stored at -20^{o}C until this step and promptly returned to the freezer. Nine μL of the resulting mixture was added to the negative control reaction tube. Next, 59μL of DNA was added to the master mix tube and vortexed for 5 seconds. Ten μL of the resulting mixture was added to each reaction tube on the plate except the negative control tube. The samples were applied above the primers, which were dried at the bottom of the tubes, to avoid cross-contamination between tubes. Once all the reagents were added to the tray, the tray was tapped gently to the bench to settle the liquids and visually inspected to ensure each reaction well had been filled before starting PCR. The Micro SSP™ D mix contained a pink/light

purple die that facilitates visual inspection. The tray was covered with a plastic tray seal and inspected to ensure each well was properly sealed close.

2.1.4.3: Polymerase Chain Reaction

The sealed tray was then transferred into a thermo cycler. A pressure pad was pressed onto the top of the tray before the thermal cycler lid was closed. The following program was used:

# Cycles	Step	Temp (^{o}C)	Time (sec)
1	1	96	130
	2	63	60
9	1	96	10
	2	63	60
20	1	96	10
	2	59	50
	3	72	30
End	1	4	---

Once the program had been selected, a 10μL reaction volume option was selected, and the PCR program was started. Each PCR program took approximately 1 hour and 16 minutes and the last step held the plate at 4^{o}C until it was removed. In some cases, the tray was stored at -20^{o}C until the electrophoresis procedure.

2.2.4.4: Gel Electrophoresis

The gel electrophoresis step involved preparing a gel, transferring the contents of the tray into the gel, running the gel, and visualizing the results.

The kits required that a 2.5% agarose gel containing 0.5μg/mL EtBr be made for each kit. Gels were made fresh immediately prior to use by combining 30mL 1x TBE with 0.75g agarose powder in a 500mL Erlenmeyer flask. The contents of the flask was swirled to mix and heated in the microwave to create a homogenous solution. The flask was heated until bubbles formed, and then removed from the microwave and swirled. This was done three times, at which point a homogenous solution was created. Then, 15μL EtBr was added and swirled to mix. The liquid was then poured into the gel dock and bubbles were removed. The combs were then fitted into the gel and the gel was allowed to set for 15 minutes. The combs were removed and the gel and gel electrophoresis buffer was poured over the gel such that the entire gel was covered by the buffer.

Following PCR amplification, the tray seal was gently removed and care was taken not to splash the samples. A multichannel pipette was used to transfer the tray contents to the corresponding wells in the gel. The gel was then electrophoresed at 140-150 volts until the red tracking dye migrated about 0.5cm into the gel (3-5 minutes). The gel was then transferred to the UV transilluminator and photographed with both regular and contrast lighting.

2.2.4.5: Analysis

The One Lambda HLA Fusion™ Software 2.0 was used to analyze the transilluminator photographs. The software was installed onto a PC with Windows 2010. The most recent reference catalog files were downloaded and updated onto the program. This

analysis program suggests allele pair assignments and required the user to make the final assignments.

The analysis software included a test gel pane, which allowed the user to manually enter the banding result pattern. The program allowed the user to enter a positive reaction, a negative reaction, and also a "no clear amplification" reaction. Once the banding pattern was entered, the program suggested allele pair assignments. Low resolution typing results were available from CBS, which facilitated the simple and quick identification of allele pairs. Typing results were transferred into a Microsoft Excel spreadsheet.

2.2.5: LABType® Sequence-Specific Oligonucleotide (SSO) typing

Following the ambiguous typing results of the SSP typing results, OneLamda offered to complete confirmatory SSO typing of the 47 samples that had been SSP typed. Forty μL aliquots of DNA samples, as well as the original gel electrophoresis images, were sent to OneLambda.

Like SSP typing, SSO typing involved a PCR step to amplify target DNA with a group-specific primer. The resulting PCR product, which had been biotinylated, was denatured. Complementary DNA probes that had been conjugated to fluorescently coded microspheres were then able to hybridize with the biotinylated and denatured PCR product. The microsphere mixture that was used had a set of fluorescently labeled

microspheres with unique sequence-specific oligonucleotide probes that corresponded to HLA alleles. The microsphere mixtures also had negative and positive control microspheres so that non-specific background signals could be subtracted and raw data adjusted for variations in sample quantity and reaction efficiency. A flow cytometry analyzer identified the fluorescent intensity of the phycoerythrin (PE) bound to each microsphere, and HLA allele assignments were made by comparing the reaction patterns with published HLA gene sequences.

Tests were completed by OneLambda technicians following LABType® SSO Typing Tests Protocol. The original SSP images and a spreadsheet with allele assignment results were received from OneLambda, and results were compared to original SSP typing results.

2.2.6: HLA Sequencing

A total of 167 samples (125 samples from 2007-2008, and 42 of the samples from 2008-2010 that were SSP and SSO typed which had sufficient aliquots left for direct sequencing) were sent to the National Microbiology Laboratory for direct sequencing of the HLA-B locus. Direct sequencing involved a PCR step to amplify the HLA Class I and Class II genes followed by agarose gel electrophoresis,

2.2.6.1: Amplification of the HLA Class I and Class I Genes:

2.2.6.1.1: Reagents

2X mix: 120mM Tris-HCl (pH 9.0), 3mM $MgCl_2$, 30mM $(NH_4)_2SO_4$, 200μM dNTPs, 0.2% gelatin, ddH_2O. Total volume 45.5mL

PCR master mix: 22.75μL 2X mix, 13.75μL Taq polymerase, 55uL each of both forward and reverse primer (previously diluted to 24μmol with Tris-EDTA buffer).

6X loading buffer: 30g glycerol, 0.25g bromo powder, 0.25g xylene powder

1X TBE buffer: 21.6g Tris base, 11g boric acid, 80mL 0.5M EDTA (pH 8.0). Dissolved in ddH_2O for a total volume of 2L

1% Agarose Gel: 1% agarose (Invitrogen UltraPure™ Agarose) in 1X TBE buffer and 0.03% EtBr

Table 2.1: HLA-specific PCR primers with corresponding melting temperature TM and annealing temperature (Ta) in ^oC

HLA gene and primer name	Primer Sequence (5'-3')	Tm (^oC)	Ta (^oC)
BPCRF	GGGAGGAGCGAGGGGACCGCAG	71.3	66.3
BPCRR	GGAGGCCATCCCCGGCGACCTAT	69.1	64.1

2.2.6.1.2: Polymerase Chain Reaction

The DNA samples were amplified using a reaction mix made of 20μL autoclaved ddH_2O, 5μL DNA, and 25μL master mix for a total of 50μL per well on the plate. The plates were centrifuged for 10 seconds at 1000rmp to make sure that the samples moved to the

bottom of the plate wells. The plates were then placed into the DNA Engine Peltier Thermal Cycler™ (Bio-rad). The following program was used:

# Cycles	Step	Temp (^{o}C)	Time (min)
1	1	96	5
44	1	96	1
	2	66.3	1
	3	64.1	1
	4	72	2
1	1	72	10
End	1	4	--

2.2.6.1.3: Agarose Gel Electrophoresis

The electrophoresis step was used to determine if the PCR amplication of specific HLA Class I and Class II genes was successful. For each PCR product, 5μL of PCR product and 2μL of 6X loading buffer were mixed together and loaded into a well of the 1% agarose gel. A 1kB ladder was also added to the first well of each row of the gel for size reference. The gels were placed into electrophoresis tanks that were filled with 1X TBE. The gel system was programmed to run at 120 volts for approximately 50 minutes. To visualize the resulting banding pattern, the BioRad™ UV Transilluminator 2000 and BioRad™ Quantity One® program were used.

2.2.6.2: Purification of PCR product

The post-PCR purification step was performed by the DNA Core Facility at the National Microbiology Laboratory. The Agentcourt® AMPure protocol system combined with the Beckman-Coulters Biomek FX system was used. The process involved using magnetic beads which bind to PCR amplicons. A magnetic field was then applied to separate out

the desired PCR amplicons away from contaminating reagents and the contaminants were then washed out using an ethanol step. The final purified PCR product was then eluted in TE buffer. Only samples that showed good banding patterns were used for sequencing

2.2.6.3: Di-deoxy Chain Termination Sequencing of HLA Class I and II Genes

2.2.6.3.1: Reagents

Table 2.2: HLA specific PCR primers with corresponding melting temperature ™ an annealing temperature (Ta) in ^{o}C

HLA gene and primer name	Primer Sequence (5'-3')	Tm (^{o}C)	Ta (^{o}C)
BSEQ5F	GGGGACGGGGCTGA	58.8	53.8
BSEQ3R	GGATGGGGAGTCGTGACCTG	64.0	59.0

DNA precipitating solution: 5mL 95% ethanol, 250μL sodium acetate

2.2.6.3.2: Sequencing PCR

Sequencing PCR required that the forward and reverse primer reactions be done separately. The reaction mixtures consisted of 220μL Applied Biosystems ™ BigDye® Terminator, 4μL purified DNA, and 165μL of appropriate primer. Two types of BigDye® terminator were used: V1.1 was used for all HLA typing except for HLA-A, for which V3.1 was used. Both Terminators used fluorescently labeled dideoxynucleoside triphosphates (ddNTPs) as chain terminators. The sequencing protocol used was based on Sanger sequencing which uses the fluorescently labeled ddNTPs as chain terminators since they lack the 3'-hydroxyl group. The 3'-hydroxyl group was required for DNA polymerase to add the next dNTP. The following PCR sequencing protocol was used:

# Cycles	Step	Temp (^{o}C)	Time (sec)
1	1	96	180
79	1	96	30
		53.8	30
		59	30
		60	240
End	1	4	--

2.2.6.3.3: Ethanol Precipitation and Sequencing

Following the sequencing PCR step, the DNA was precipitated with 21μL DNA precipitating solution. The plates were placed in the dark for one hour at -40^{o}C, and then centrifuged for one hour at 4000rpm at 4^{o}C to precipitate the DNA. Next, the supernatant was discarded and the plates were inverted and spun for 30 seconds at 500rpm to remove ethanol. Next, 130μL of 70% ethanol was added to each well and the plate was centrifuged for ten minutes at 4000rpm. Again, the supernatant was discarded and the plate was inverted and spun at 500rmp for thirty seconds. These steps were repeated one more time in an effort to eliminate any remaining salts and reagents from the DNA pellet. To remove ethanol residue by evaporation, the plates were incubated (uncovered) on a thermocycler at 90^{o}C for one minute. Next, the DNA was re-suspended in 20μL of Hi-Di™ formamide (Applied Biosystems), which disrupts the hydrogen bonds between DNA strands and promotes strand separation. To denature the DNA into single strands, the plates were covered with foil and incubated at 90^{o}C exactly for 90 seconds. Following this step, the plates were immediately placed on ice to prevent the DNA from re-annealing, and the contents of each well was transferred to MicroAMP™ sequencing plates and sequenced in the 3130xl Genetic Analyzer (Applied Biosystems)

2.2.6.4: Genotyping

The output sequence information results from the 3130xl Genetic Analyzer (Applied Biosystems) was in the form of electropherograms. The analysis software CodonExpress™ was used to analyze the electropherograms. The genotypes of each HLA loci were assigned by comparing the sequences to known alleles for each loci found in the IMGT HLA database from the European Bioinformatics Institute (45).

2.2.7: Subset Analysis

Clinical information was obtained from five patients from Manitoba through a review of medical charts. Patients information regarding $CD4^+$ T cell counts and viral load as well as other relevant clinical and personal data since diagnosis with HIV was available for patients alive and under care. Charts were obtained from the Manitoba HIV Clinic and searched for important information.

Chapter 3: Solvent Use and HIV in Manitoba

3.1: Rationale

The hypothesized link between solvent use and HIV is largely supported by observations from individuals who work closely with the population and anecdotal evidence from local health practitioners. Two distinct long-term research directions provided the rationale framework for this study. These state that HIV risk, in the context of solvent use, is affected by biological as well as social factors.

Regarding the biological rationale, the research team hypothesized that solvent use damages mucosal surfaces and leads to increased microbial translocation and subsequently higher levels of immune activation. Additionally, it was hypothesized that solvent use impacts the natural killer subset of cells. Both of these mechanisms may have important consequences for HIV risk. Regarding the social rationale, the research team hypothesized that solvent users experience social inequalities, such as discrimination and exclusion which result in many unmet needs. Additionally, it was hypothesized that solvent users may be involved in risky behaviors that may increase HIV risk.

This project aimed to investigate the link between solvent use and HIV through a multi-phase interdisciplinary research project. To address both research directions, the intention was that phase one would be a community-based-research focused research

study which would inform phase two, a basic science research study. This chapter presents work from phase one of the project which consisted of two main objectives and four major research questions, outlined below.

3.2: Objectives

1. Establish a research partnership with Sunshine House
2. Characterize what solvent use is and who solvent users are in the context of the Winnipeg solvent-using population through focus groups with solvent users and individual interviews with solvent users and key informants

3.3: Major Research Questions

1. What is solvent use in the context of Winnipeg, Manitoba?
2. Who are solvent users in the context of Winnipeg, Manitoba?
3. To what extent is the research team and approach accepted by the solvent-using population?
4. To what extent is a second phase of project, involving biomedical research, feasible?

3.4: Results

3.4.1: Study Population

Three focus groups were completed with solvent users. The first focus group was a men's group with five men in attendance. The second group was a women's group

which three women attended. The final focus group was a mixed group and in attendance were two men and one woman. Nine individual interviews were conducted with solvent users; six men and three women were interviewed. Participation in a focus group did not result in exclusion from participating in individual interview because the nature of the questions used in the two interview types was different.

All participants were adults and identified as Aboriginal. Demographics of the focus groups (n = 11) were as follows. The age range of men was 33-55, with an average age of 46. The age range of women was 24-44 with an average age of 35. Three participants identified as Treaty and two participants identified as Dakota/Ojibway. The remainder of participants were split between the following self-identifiers, with each occurring once: Cree, Soto/No Treaty, Cree/Soto, Ojibway, Aboriginal/Non Treaty, and Métis.

Six individual interviews with key informants were completed. The demographics of the key informants are as follows: four key informants were service providers, one key informant was a physician, and one key informant was a researcher.

3.4.2: Qualitative Results

The study findings that resulted from three focus groups with solvent users, nine individual interviews with solvent users and six individual interviews with key informants are presented below. The findings are divided into six sections with the following themes: description of solvent users, description of solvent use, infectious

diseases, reactions & stigma, relationship between service providers and solvent users in Winnipeg, and research. Throughout the text, solvent users are identified as "participants" and "solvent users", and are referred to by gender, where appropriate. Key informants are identified only as "key informants."

3.4.3: Description of Solvent Users

We aimed to characterize solvent users in Winnipeg. We asked questions around their histories of solvent use, and other drug use as well as their reasons for using solvents. Relationships and social networks emerged as an important theme throughout interviews. We also inquired about physical and mental health status. Key informants were also asked to describe the solvent using population.

3.4.3.1: History of Solvent Use

We asked individual interview participants to talk about their histories of solvent use. All participants had their first experience with solvents between the ages of 9 and 16. Four participants were introduced to solvents at the encouragement of siblings or cousins, four were encouraged by friends, and one participant described watching a brother use solvents and later trying when alone. The first solvents that participants used were evenly split between Cutex nail polish remover, lighter fluid, gasoline and lacquer thinner. While all participants currently use lacquer thinner, eight had histories of trying or regularly using additional solvents following initiation. Four participants used plastic wood (a product typically used to fix imperfections in wooden surfaces), three used

contact cement, two used LePage airplane glue, two used spray paint and one used Liquid Paper. One participant described solvents other than lacquer as “kids stuff” and said, “I wouldn’t go sniff plastic wood or airplane glue or Cutex or anything like that now, at my age.” Generally, solvent use was episodic between initiation and current use. As kids/teenagers, participants tended to steal solvents or pitch in money together and use with friends in the bush, in the park, at local hangouts or at a friend’s house. Two participants specifically mentioned that it was not a habit at that time in their lives. All participants described breaks from solvent use for a variety of reasons including moves, school, jail time, detox/treatment program, and getting involved with other drugs.

3.4.3.2: Other Drug Use

During the individual interviews, participants were asked about other substance use. All participants had a history of using marijuana, with seven participants using marijuana ranging on a spectrum between regularly to “once in a while”. Most participants had histories of alcohol use and self-described alcoholism. Of the five individuals who reported currently using alcohol, four included the caveat that they do not drink heavily. Six participants reported ever using crack-cocaine, with three participants continuing to use crack-cocaine. Regarding crack-cocaine use, most participants described smoking it and none specifically indicated injecting crack-cocaine. Five individuals had at least one experience with injection drug use, and one participant injects regularly. Four individuals had a history of injecting Ts and Rs. Ts and Rs, Talwin and Ritalin, also called

a “set”, are a combination of injectable drugs. Talwin (Pentazocine) is a painkiller, and Ritalin (Methylphenidate) is a stimulant. They have been described as being similar to heroin mixed with cocaine, and have been called “poor man’s heroin” (78). Indeed, several key informants mentioned an association between solvent use and injection drug use. Four participants described recreational prescription pill usage, with one participant continuing to use prescription pills (Diazapam). Five individuals reported using psychedelics (mushrooms, acid) previously in their lives, and three participants reported ever snorting cocaine. Regarding mixing experiences, one participant reported using solvents and injection drugs concurrently, and one participant fell down the stairs and was admitted to the emergency room following mixing solvents, injection drugs, and alcohol.

Participants were asked which substance they would use if they had their choice. Three individuals chose solvents, and one person could not decide between solvents and marijuana. Two participants chose alcohol, and other choices included Ts and Rs, and crack-cocaine.

3.4.3.3: Reasons for Using Solvents

During the individual interviews two men expressed that boredom was a reason that they use solvents. They remarked that “there’s nothing to do on the street”. One man talked about “the excitement of having a rag full” and procuring solvents and the other

said he used solvents “just to kill the boredom.” Both men mused that something that kept them occupied like school or a job or the gym would improve their lives.

The low cost of solvents was a reason that most participants chose to use solvents. It was noted that $5 worth of solvents would last a person all night, and that an amount described as a “fill” or “soaky” could be purchased for $1, by trading in cigarette butts, or by mooching. Alcohol, cigarettes, Ts and Rs and crack were noted as being more expensive in comparison and not as long-lasting, which was illustrated by one woman saying, “Sniff is a lot cheaper than the rest of the drugs. That’s why I think a lot of people are doing that,” and, “it’s cheaper and it lasts longer.” Participants demonstrated a sense of pride around not depending on methods such as stealing, panhandling and prostitution, to obtain money to buy solvents. One man said, “We’re not bank robbers or anything.”

The use of solvents as a coping mechanism emerged as a theme from both the individual interviews and focus groups. Indeed, histories involving trauma and loss became apparent through the course of interviewing solvent users. Exclusion from families, death of loved ones, and the apprehension of children by Child and Family Services were common. A focus group participant illustrated this point by saying, “When I walk around and look at these people, it seems like we all grew up the same way… most of us… like me and him, and her… The things we loved got tooken away from us.” One woman talked about losing her children due to solvent use and said, “I lost a lot of

respect towards myself." Incidents of violence and accidents requiring hospitalization were also common. A striking example of violence was that one man was covered in solvents and lit on fire after passing out. Seven participants from the individual interviews described using solvents as a coping mechanism, and four specifically referred to solvents having a mind blocking effect. One woman said, "it takes away my [emotional] heart problems," and participants in the men's focus group agreed when one man said, "That's why people sniff, to take away your worries." Several key informants spoke to the prevalence of major traumas that many solvent users have experienced, and commented that they believed solvents were being used as a coping mechanism.

A variety of additional reasons for using solvents also emerged. Participants described using solvents recreationally to relax or have fun and one man said, "I have my room, my queen sized bed, my ghetto blaster, my mix tapes and I just sit there and listen – my fire escape, with my window open – I just listen to music and just get high." One woman, who has Hepatitis C, explained that she could no longer drink alcohol because of liver damage or use crack-cocaine because her physician cautioned her that she may lose her vision. One participant pointed out that alcohol causes aggressive behavior so he opts for solvents instead. One woman said she preferred solvents to alcohol because she could snap out of the effects of solvents within minutes if she were violently attacked. One man explained that he uses solvents to pay homage to a deceased sister's memory.

3.4.3.4: Relationships and Social Networks

Participants completing individual interviews were asked about their social networks. Friendships, in general, emerged as significant relationships and friendships with other solvent users appeared especially important. While reminiscing, one participant said, “It seemed like everybody I knew was into that lacquer” and talked about using solvents within a group setting and also while going on walks, sentiments echoed by several other participants. Another participant described his friends as family and said, “they all sniff lots.” He went on to say, “Sniffers. That’s who I’ve been hanging around with all this time.” A man from the men’s focus group said, “When I hang out with my street crew, they smell just like me, nice and dirty and stinky, and I’ve got to pull my weight when I’m out there.” The tight knit and loyal nature of participants and their friends was illustrated by participants’ accounts of looking out for and protecting one another. Key informants often spoke about the tight knit and communal nature of the population.

Relationships with family were often described as strained, with parents, siblings, children, grandchildren, nieces, and nephews generally reacting negatively to solvent use. Four of the five individuals who talked about kids had the experience of their children being taken into care. Three participants talked about their adult children limiting contact with grandchildren specifically because of solvent use and one participant stated, “I used to spend lots of time with my grandkids, and all my kids, and since this sniffing started again its cut right down.” Although most participants described strained relationships with family members, two participants recalled using

solvents as adults with their adult siblings and described the usage during that time as a "habit" and "solvent abuse". One man reflected very fondly on the time he spent with his sister using solvents recreationally.

Relationships with significant others emerged as important and influential. Seven participants had current partners and many described these relationships as monogamous or long term. Two men and two women described sniffing with current or previous partners, and one woman talked about "being with other guys where sniff was kind of a normal thing". Several participants talked about partners influencing them to stop or cut down on using solvents and other drugs or alcohol, and reflected on that influence as being positive or helpful.

3.4.3.5: Physical and Mental Health Status

We asked participants from the individual interviews to rate their physical health on a scale of excellent – very good – good – fair – poor. Four participants said "good", three said "fair", one said "very good", and one said "poor." Participants from the focus groups and interviews indicated concern regarding their own health. One woman said, "I think about the ones down on Main Street and I start feeling bad. Like, I don't want to be like that." Most people reported regularly visiting a physician for check ups and blood tests. A lack of information about the long term effects of solvent use was apparent, with one man saying, "We didn't know it's going to bugger up your nerves and everything like that. There was no teaching on that, so we kept on sniffing it." In

addition, a woman remarked, “I’ve been reading, there is solvent abusers that do die. Like, I didn’t know that you could die from solvent, from all that.” One woman had had a stroke and thought that using solvents had contributed. Key informants regarded solvent users as being in poor health, and many noted that there was a lot of death in the population. In terms of non-infectious disease, key informants reported malnutrition and diabetes as other health issues.

Participants were also asked to rate their mental health on the scale of excellent – very good – good – fair – poor. Six rated themselves as “good” or “very good”, and three rated their mental health as below “good”, although it was clear that some participants misunderstood the question and thought they were being asked to rate intelligence. Perhaps a more accurate portrayal of the mental health of participants arose from the self-described usage of solvents as a coping mechanism by the majority of participants. Two male participants talked about their own experiences with suicidal thoughts, and throughout the focus groups and individual interviews several people spoke of solvent users they knew committing suicide. Beyond drug use, participants described other coping mechanisms like visiting an elder, participating in a sweat lodge ceremony, and talking to loved ones or trusted support staff about problems. Many talked about living day-to-day, “surviving,” the uncertainty of the future, and their worries about leaving loved ones behind. One man said, “If you think positive, you’ll get through with your day. That’s my theory. Think positive all the time. Never think negative or else you won’t get through in life.” Indeed, key informants spoke of solvent users living day to day

versus thinking long term about their futures, and one key informant said, “They don’t seem to have much hope for the future.”

3.4.4: Solvent Use

A priority of the research was to define solvent use practices and obtain a representative snapshot of the impact solvent use has on the body, mind, behavior, and health and wellness.

3.4.4.1: General Description

We asked individual interview participants to talk about their current sniffing practices. All participants said they currently use lacquer. Three participants said they hold a lacquer-soaked rag outside of their mouths while inhaling and one man said, “Some of them have the rag right inside their mouths…. They tell me oh, you get more of a high out of it.” Two participants said that, if they had solvents, they would start using as soon as they woke up, and one participant preferred using solvents at night because the days are too hot. A $5 “chubby” (a small bottle of lacquer) was said to last 3-4 hours to an entire night. One woman said, “With sniff, you sniff that and you put your rag away then 3-5 minutes later, you get some cool fresh air, have a glass of water, and you’re back. You know what’s going on around you.” Reasons for ending a session of solvent use included finishing a jar, deciding to be done, and feeling sick or getting the shakes. Four people talked about using solvents at home, and five specifically mentioned Main Street as a place to gather and sniff with friends. Many talked about sniffing outdoors at

various locations such as the park, under a bridge, at “BJ corner”, and while wandering or on walks. Most participants talked about using solvents in groups with friends, and other dynamics included using with one other person and using alone.

Frequency of use fell on a spectrum between a few times a month to every day, all day. Eight people recounted a history or current usage of at least once per week, and five had, at some point, used solvents every day. Many participants indicated that they sometimes had felt addicted to solvents while other times had felt in control. One man described a period of time where he didn’t crave it, followed by a period of time where it was “habit forming.” Another man said it was a “bad habit” but that he wasn’t “grinding for it” and one woman stated, “I can stop myself.” Regarding quitting solvent use, one man said, “I wish I could get off it, stay off it,” and one woman indicated that she would like to stop, but had too many problems.

3.4.4.2: The Impact of Solvent Use on the Body and Behavior

When describing the effects of solvent use, different categories of intoxication emerged. Three participants recounted times that they hallucinated while using solvents, four described a mind blocking effect, two described it as a nerve calmer or relaxant, and one described it as “like an acid trip.” Two men mentioned that they don’t get as high from it as they used to.

Participants were asked how using solvents affected their sex lives. Six participants said they do not have sex or think about sex when high, and two men said that it depended on the company that they were with. One man wondered, “I always thought, maybe, like the amount of sniffing I was doing, maybe that did something to me,” and another man said that using solvents “probably just kills your desire.” One woman said, “I think it just lowers the sex drive.” On the other hand, one man said that using solvents “makes it [sex] last longer.” One key informant and former solvent-user, said, “From my experiences, solvents were an aphrodisiac.”

We inquired about eating habits during and after using solvents. Seven participants said that they lose their appetite and do not eat while using and five went on to describe significant weight losses. One man said, “that stuff probably kills my appetite,” and another man said, “Sometimes I just go a whole day without eating, but then I’ll go eat lots the next day.” Five participants mentioned that their appetite returns when they are done using, or the next day, and many described having big appetites when not using. Two men said that they do get hungry while using solvents. Most participants mentioned maintaining a regular intake of fluids while using was important to combat dehydration and many described losing fluids due to vomiting.

Participants talked about whether or not solvents induced aggressive behavior. Four participants said that they can get aggressive if provoked, and three participants said

they do not get aggressive when using. Eight participants described themselves as people who are “quiet” or like to keep to themselves when using.

Three participants said that they are less likely to notice cold temperatures while under the influence of solvents, which may have harsh consequences given the cold winter climate of Winnipeg. Indeed, one key informant mentioned that they know of solvent users freezing to death.

Participants were asked questions around how using solvents impacts health and well-being. Five participants said they had experienced nausea and vomiting fluids or foam following heavy use and most noted the importance of staying hydrated. One participant said, “It does give you the runs now and then.” Excessive use resulted in headaches for one man. Two men said that solvent use impacted breathing and one remarked, “If I get high too much, then I won’t be able to breathe properly.” Two women talked about coughing and spitting up phlegm when using solvents. Four individuals indicated that they didn’t think sniff affected their ability to sleep. In terms of general wellness, several participants noted feelings of weakness and one woman said, “The sniff is bringing me lots down. It’s doing lots to my body and I know it’s affecting me now.” Two participants described wobbly legs and shakes. It was noted that other solvent users have incurred mobility issues and several participants described measures they take to avoid a similar outcome such as regular stretching, eating regularly, and stopping solvent use once limb shaking begins. Three participants talked about changes

to skin. One man described sweating out a gummy, sticky substance, one woman said that solvent users get white patches on the skin where the solvent comes out of pores, and one woman had dry skin on the hand that holds the rag. Three individuals mentioned a hangover and two described them as worse than an alcohol-induced hangover.

3.4.5: Infectious Diseases

We asked solvent users about their experiences with HIV, and other infectious diseases like Hepatitis C, tuberculosis, influenza and colds. We also probed about risk behaviors around routes of transmission. Key informants were asked to describe their experiences with infectious diseases within the solvent using population.

We asked participants of focus groups and individual interviews if they felt concerned about HIV. Many were regularly tested, but one man said he was never tested. None of the participants disclosed a positive HIV status, and many talked about friends that had it. One woman said that she did not know anyone who had HIV. Some participants demonstrated that they knew information about HIV transmission and that they pass on the information to others. Some participants were lacking in basic knowledge, and two individuals suggested that HIV could be transmitted through saliva. Indeed, key informants acknowledged that there was a lack of education regarding HIV. Generally, key informants believed solvent users to be at high risk for acquiring HIV, that HIV was present at high rates in the population, and that solvent users did not view HIV as a

larger concern than any other health or social issue. One person said, “I think HIV is ready to explode in that population.” An interesting observation surfaced from one key informant who said that they knew of four or five cases where solvent-using HIV sero-discordant couples, despite sharing needles and not using condoms, were not exhibiting HIV transmission. The key informant asked, “Is there something about solvents that has an instant anti-retroviral effect? Like, that it can kill HIV in the body, because how are these people not infected?”

Solvent use addiction was noted as a barrier to HIV and Hepatitis C treatment, with one key informant saying, “They’ll probably never be able to treat the Hep C in this type of setting unless they really get control of some of their addiction issues.” Regarding the challenge of adherence to medications, a key informant talked about bubble packing HIV medications with “Tylenol 3s or other medications that they find useful like Benzodiazepines or Valium,” as incentives towards adhering to HIV medications.

Participants appeared to be more concerned with Hepatitis C than HIV and seven individuals knew their Hepatitis C status. Of the three participants who disclosed that they had Hepatitis C, two thought they acquired it through blood transfusions, and one felt they acquired it by sharing spoons/filters/water, but not needles, during intravenous drug use. One person had undergone treatment for Hepatitis C and the other two had physicians that did not recommend treatment for different reasons. One man said, “He [the doctor] wants me to start treatment, but he wants me to stay clean,” which

indicates that some physicians view sobriety as a necessary step before Hepatitis C treatment can begin. A woman said she had not done treatment because “when they did do that biopsy, they said that my liver is looking good.” Key informants demonstrated that they were aware that Hepatitis C was present at high rates in the population.

Key informants described solvent users as being a “catching point for every marginalized infection,” including tuberculosis and sexually transmitted and blood borne infections (STBBIs) like syphilis. Regarding other infectious disease, two participants had histories of tuberculosis. Most participants reported that they did not get the flu or colds or very often, and several did not have a cold or flu in the previous year. On the other hand, one key informant suggested that solvent users are much more prone to catching colds and the flu and mentioned that solvent users are also often seen in clinics for soft skin infections.

We asked participants if they were taking precautions regarding avenues of disease transmission, such as sexual contact and intravenous drug use. Generally, precautions like condom usage and avoiding needle sharing mitigated concerns. One man said that he uses condoms every time he had sex to avoid unwanted pregnancies, and another said he will use condoms at a woman’s request. Both men said that using solvents did not make them less likely to use condoms. Several participants explained that they did not use condoms because they were in monogamous relationships with trusted

partners. One woman said that she has had sex with people that are HIV positive without using a condom, and that she never uses condoms. Many participants demonstrated that they knew not to share needles, but it seemed like sharing other intravenous drug use tools like water, spoons, and filters was still occurring. One woman expressed concern over catching Hepatitis C from scratches or bites incurred during fights. Most key informants thought that solvent users were more likely to be having sex without condoms and more likely to be using and sharing needles when using injection drugs.

3.4.6: Reactions and Stigma

Reactions and stigma towards solvent use and solvent users emerged as an important theme throughout the individual interviews, focus groups, and key informant interviews. Participants talked about the largely negative reactions they experienced during youth, and also throughout adulthood from family, significant others, other drug users and the general public.

3.4.6.1: Reactions During Youth

Participants reflected on reactions from friends and family during youth. Many talked about taking measures to prevent parents or siblings from finding out about their solvent use, such as hiding in the bush, hanging out at friend's places, or going for walks after using so the smell would fade. Generally, parents disapproved and several people mentioned siblings or cousins disapproving, unless they were engaging in solvent use

too. One man said that many people in his family, including his mother, were using solvents throughout his teenage years. Another man spoke about people on his reserve finding out and telling others that he was using solvents, and a few individuals talked about being kicked out of home or running away. A woman spoke about some boyfriends not liking the smell, and that, with other boyfriends, using solvents together was "normal."

3.4.6.2: Reactions as Adults

Participants talked about reaction and stigma they have encountered as adults. Many participants spoke about being the only person in their family to currently use solvents and described the negative reactions of their families. Three participants said that their solvent use prevents them from visiting with their grandchildren because their grandchildren's parents disapprove. Participants in the men's focus group discussed being the "Black Sheep" of their family, and about how that reaction bothers them. One man described being kicked out of his family specifically because of his drug of choice despite his family using other types of drugs. Three women and one man talked about people reacting negatively to the smell using solvents imparts on their clothes and bodies, and one woman said, "They smell it on me, like after I'm done like, a couple of days after or something. It's because it's in my lungs." A woman described her high-school aged children's reaction, "They're disgusted," she said, "They say I look like some kind of Ethiopian or whatever, from a third world country." One man said his family encourages him to enter treatment programs, and one woman implied that her

daughters encourage her to visit for weekends in an effort to keep her from "doing the bad stuff."

Significant others generally disapproved of their partner's solvent use, but many participants described having relationships, at some point, with another solvent user where solvent use was encouraged. Several men talked about their partners influencing them to quit or cut down. One man said he didn't think it would work out if he were to date a girl that also didn't use solvents, and another man admitted that he hides his solvent use from his girlfriend. In the men's focus group, there was a discussion around being blamed if a girlfriend were to die, with one man saying, "You're the sniffer, you're supposed to be watching." One man said, "I had kids with a couple of girls… Then, they wouldn't stop [using solvents] so I ditched them."

It became clear that solvent users were considered to be low on the drug using hierarchy by other drug users, and one key informant described solvent users as "bottom of the barrel." One woman said, "I know it's a drug but it's not a regular drug that you would really… spend a lot of time on," and many other participants admitted that they felt embarrassed about their drug choice sometimes. The women's focus group talked about being judged by people who drink rubbing alcohol, hand sanitizer or hair spray. "We're all in the same boat," said one woman, a sentiment echoed by the two other women in the focus group. One man talked about his frustrations with his

family who judge him harshly for solvent use while they used other drugs he considered just as bad or worse. He said, “They’re more zombies than I am.”

Participants also described reactions from the public. One man explained that he “doesn’t do it [use solvents] publicly because of peoples’ opinions or what they think,” and another man said that it doesn’t bother him that people react that way. One woman that said she feels people see solvent users and think, “Look at that person. That’s nothing but a sniffer,” and she went on to say, “We weren’t born with that rag in our hand, or that pipe in our mouth, “ and, “we’re not just useless people… because we’re users, we’re sniffers. Like, we’re humans, just like them.” Participants from the mixed focus group talked about “half the cops in the city“ being prejudiced towards Aboriginal people. One woman said, regarding the media’s position on Aboriginal people, “They don’t even give a care when it comes down to Indians.” Key informants described the solvent using population as alienated and marginalized. Regarding drug users more generally, one key informant said, “There might be solvent use history there or solvent use may be part of the spectrum of using, but people don’t talk about it. I think its really stigmatized,” and, “The most needy don’t get the right services or even get any services, so its our society [that] really stigmatizes drug use generally, but also people who use solvents are even more stigmatized.”

3.4.7: The Relationship Between Solvent Users and Services in Winnipeg

Many of the key informants we interviewed had years of experience working with solvent users in Winnipeg. We asked them to talk about their experiences engaging with the population, and to describe barriers and challenges that they encountered along the way. A snapshot of the current climate of services in Winnipeg emerged from the three sets of interviews. Key informants spoke candidly regarding organizations that accept or exclude solvent users. Places that were generally regarded as being accepting and respectful towards solvent users were Main Street Project, Sunshine House and Mt. Carmel Clinic's SOS program.

3.4.7.1: Successful Engagements, Barriers, and Challenges

Key informants shared accounts of what they considered to be successful engagements and relationship building between service providers and solvent users. Several key informants spoke of the long-term nature of their positions as an avenue to gradually become familiar with, and earn the trust of, people who use solvents. One key informant said that sharing personal information, such as their own history of solvent use, their HIV status, and their Aboriginal status, was a way to connect with people. Many key informants identified themselves as being advocates for the population. Key informants also spoke to the perceived challenges of working with solvent users, and key informants talked about addressing the barriers between staff and clients by hosting meet and greets where staff could "see the people first, and the drug of choice second." Regarding communication issues, a key informant said they have learned that "it's

almost like working with people who are FASD [Fetal Alcohol Spectrum Disorder]. So you have to be very repetitive, you know, they don't quite understand all the time the consequences of their actions, so they know stuff, but they can't quite put it into action."

We asked key informants to talk about the barriers and challenges they have encountered while working with solvent users. The most frequent challenges reported were that solvent users tend to live day-by-day versus thinking long-term, that solvent users can be difficult to communicate with while intoxicated, and the lingering odor of solvents on the clothes and bodies of solvent users. One key informant said that service providers "automatically smell solvents" and assume that "they're all cognitively damaged" and further assume that "they don't know what they're doing, that there's no point in helping them," and said they thought these assumptions were not true. Key informants often referred to social issues related to homelessness and poverty as barriers. One key informant talked about solvent use being unique to Winnipeg, and said, "It's really a Winnipeg issue and really an issue with the inequalities of health and the poverty that our city is facing and the challenges that we're going to face as a city." Key informants also mentioned political barriers, such as the funding environment with a Conservative government, fighting through the red tape of organizations that exclude solvent users, and a police chief (at the time of the interviews) that is not supportive of harm reduction. One key informant recalled that, in the late 1980's, there was a closure of a needle exchange due to solvent users frequenting the facility more than expected.

Many key informants spoke about the limitations of their own fields, and expressed that the lack of organized help results in people "falling through the cracks." Key informants acknowledged not having an appreciation of what the main concerns of solvent users are. One key informant succinctly said, "We don't understand enough about them or what kind of services they need."

3.4.7.2: Health Care

Key informants spoke about issues around access to primary care. One key informant said, "They get, quite often, very poor medical care… Either they can't get in to see a doctor or the doctor figures that they're just not compliant because they're not taking their medication or following through with what's been expected of them," and added that health practitioners may not take into account social factors associated with homelessness, like access to shelter, meals, showers, and that things get stolen. The lack of knowledge about the long-term effects of solvent use was also noted as a challenge that health care practitioners face.

Four Rivers Clinic on Main Street was said to have a "no sniffing policy," and a key informant said, "Somebody who had been sniffing the night before, [was] denied service first thing in the morning because they still smelled like it." One key informant said that the Mt. Carmel clinic, Nine Circles clinic, and the Manitoba HIV Program clinic at Health Science Centre have been "quite open to people", but also added, "I think that they

don't have quite the welcoming environment," and noted that appointment-based primary care may not be the best approach. Throughout the individual interviews and focus groups, many participants mentioned histories of using solvents during pregnancy, or knowing women who did. Stop FAS, a program for mothers at risk for delivering babies with Fetal Alcohol Syndrome, were also said to deny services to solvent users "because they were too difficult." This has identified an important gap in primary health care services and support for pregnant women who use solvents.

Given that most participants had histories of trauma and loss, it was interesting that specific or helpful mental health services were not discussed more often. One woman talked about her experience visiting a psychologist that was very judgmental and told her that her children were better off not being in her custody and that they were going to become "drunks or crack heads," or "sniffers." Many participants said that talking to someone like an elder, a religious friend, or a service provider with whom they had a long-standing relationship was helpful. One participant said that attending sweat lodges was beneficial. It was clear that most participants had no interest in speaking to mental health professionals that gained experience exclusively from academic sources. Participants seemed more willing to talk to people who had been through similar life events or were at least willing to learn and understand them and not be judgmental.

Barriers to accessing and possessing harm reduction supplies, such as clean needles and crack kits, emerged as an important issue by key informants. Indeed, many solvent users

identified as currently or formerly using crack cocaine or intravenous drugs. One key informant said that an organization called 180 Henry (Salvation Army Booth Centre), as well as other unnamed shelters destroy harm reduction materials like needles and crack kits, and then later complain that clients do not have access to supplies. One key informant described the Downtown Access Centre as "fabulous" once you get in there, but said that entry requires standing outside the building, pushing a button to connect to the intercom, and stating "I'm here for clean needles. I'm an injection drug user." The influence of the police was clear, with one key informant saying, in July 2011, "We currently don't have a police chief that is supportive of harm reduction where there used to be a police chief who was," and, "Harm reduction is always on shaky ground. You take two steps forward, you take two steps back." Another said, "We haven't established what the primary harm is for solvent use." Notably, two categories of harms associated with solvent use were mentioned: the harms associated with intentionally inhaling solvents, and the harms associated with the unintentional inhalation of solvents by bystanders, such as headaches.

3.4.7.3: Housing/Shelter

Housing and shelter emerged as concerns throughout interviews with solvent users and key informants. During an individual interview, one man said, ""I'm homeless, eh, and I think about where I'm going to eat next and they wouldn't let you go in there if you're smelling like sniff or anything like that." The men's focus group also talked about a "sniff house" – a residence where people bring solvents as an "entry fee" to "sniff and

sleep" on the property. Key informants' accounts supported that housing is an issue for the population and many spoke about the importance of people having a safe place to live or sleep. One key informant talked about solvent users getting kicked out of housing or shelters because they smell or are using solvents, and said, "There are a lot of drug addicts out there, and they're not penalized because they're high." Siloam Mission and 180 Henry (Salvation Army Booth Centre) were identified as shelter-based organizations that exclude people for solvent use. Most participants mentioned Main Street Project as being inclusive towards solvent users, and one key informant noted that Main Street Project is a place that many solvent users identify as "home".

3.4.7.4: Drug Treatment Programs

Many participants had histories of entering treatment programs for substance use issues including, but not limited to, solvent use, with stays ranging from a few minutes to several months. Places that people went include Pritchard House, Addictions Foundation of Manitoba, Behavioral Health Foundation/St. Norbert, Alcoholics Anonymous, Salvation Army, Peguis, and many participants mentioned "detox" more generally. The absence of a treatment option tailored to the specific needs of solvent users arose as a major theme throughout all of the interviews. Many participants pointed out that treatment programs exist for alcohol and other drugs. One man said, "There's nothing for solvent abusers, and there are lots over there, and they are asking for help but they don't know how to go about doing things," and one woman exclaimed, "I need treatment, I do… But I don't know where to start. That's a problem!" Key

informants also spoke to the lack of solvent-specific treatment options, the limitations of mainstream treatment options, and the need for the development of a treatment program in Winnipeg. One key informant talked about a letter from an income worker that read, “If you don’t enter a solvent treatment program, you’re cut off.” Regarding existing non-specific treatment options, one key informant said, “A lot of those treatment programs run anywhere from 6 weeks to 3 months. My opinion, not a long enough time for someone who’s been a chronic solvent user for a long time. You know, they probably haven’t even gotten half of the solvents out of their system.”

3.4.8: Research

One of the priorities of this research was to ensure that the research team was taking an appropriate and respectful approach to current and future research with the solvent using population. As such, we asked participants and key informants to provide feedback on the project thus far, and advice for designing later phases of the project. We also inquired about how we should design the knowledge translation phase of the project and were provided with many ideas for practical applications of the research.

3.4.8.1: Feedback and Advice

We asked participants of the focus groups questions about their opinions towards various practical aspects of research. The participants of the focus groups were curious about the research and asked many questions throughout the groups. According to participants, Sunshine House was an acceptable location for participants to open up

about confidential aspects of their lives and histories. Many described Sunshine House as a comfortable place that was private. One man said, “We feel more comfortable here than we do in some other place because our background is here.” Participants were also comfortable with the research team. One man said, “It seems like you people know what you’re doing and groups can learn from you.” Another man said, regarding the interviewer, “I wouldn’t mind talking to you because you know, you know me from the first meeting. You know everything. So I think I’d feel comfortable being interviewed by you.” We asked people how they felt about sobriety being required for informed consent. Participants said that it was not mean to ask people to come sober and that it was not more difficult to talk about things when sober. One man said, “For a program like this, I think… leave the solvents at home. Everybody should come straight, so you know what you’re signing and you know what you’re talking about and understand what they are saying.” On the other hand, one woman expressed that she has to be high at all times because she cannot live her life straight.

We asked participants questions about the acceptability of later phases of research and how it should be designed. All participants said they would be willing to participate in later phases of research that involved them providing blood. One participant said, “if its going to help other people who... if it concerns them. Then no problem.” There was no consensus on which days of the week would be best for people, although some people thought Tuesday/Wednesdays and Sundays would work, and Friday was said to be “party day for everybody”. Transportation was said to be an issue during bad weather,

and one man said that transportation is an important resource that needs to be in place so that people are safe. Participants from the mixed focus group said that it was a good idea to have food, and one man said that he and his friends would be insulted if someone were to say they were only participating because of the food.

Focus group participants were asked about which types of support should be built into later phases of research and discussed both individual level support and group support options. Regarding individual level support, the majority of participants said that they would prefer to have someone to talk to that had been through similar life experiences or willing to learn about theirs. An acceptable support worker would be non-judgmental, accept them, understand their emotional problems and be "neutral" in regards to faith and spirituality. Many participants said that an elder would be an inappropriate choice because not everyone has the same beliefs, and "you're supposed to be straight and all that before you talk to them." One man thought that the men in the focus group would make good councilors since they have the life experience. Participants also had suggestions for group supports. Ideas that were expressed included expanding the SOS meetings to additional days, having a group similar to SOS meet at Sunshine House, and fun activities for solvent users. (The SOS is a group for solvent users that meets weekly at Mt. Carmel clinic.)

Key informants were asked to offer advice and cautions for working with the solvent using population. The most common advice was to be honest and up front with

participants regarding the expectations of the research team, and the limitations of research in general. “Your word is big with them,” said one key informant. Key informants also advised that the research team should treat participants with dignity and respect, and also consider them as experts on the topic of solvent use and to make it clear that their input is valued. Key informants were concerned about the population being taken advantage of, and advised that the research team should be genuinely interested and not have a paternalistic attitude.

3.4.8.2: Practical Applications of Research

Participants had several ideas for how and where results and information about solvents should be distributed. One man thought that a film would be an efficient way to disseminate information to other communities. Other suggestions included pamphlets, a commercial, billboards, and posters. Participants pointed out that these types of materials exist to educate or alert the public regarding drunk driving, alcoholism, and prostitution. Focus group participants were agreeable to having their words and information included in academic publications. One man said, “I like my words being published out there,” and many expressed that they hoped it would help other people, down the line.

Focus group participants had several ideas about how a solvent-specific treatment option should be designed. Suggestions that were made include using a small building, like Sunshine House, that has around twenty beds and having sharing circles every day

and time to have one-on one conversations with people. Another man added that, “for the abusers, they could stay there for maybe a week or two weeks,” with regular follow-ups if people were falling behind. One woman said there “should be like 30 days or 3 months programs for this,” and that people that know more about solvent use should be involved. Another woman said there should be a group like “Solvents Anonymous” in a big gym with chairs. The men also included restrictions and rules that should be in place, like “you can’t sniff while [in the program] we’ll let you go, you’re gonna come back, you have to be off that stuff.” Key informants also talked about things that should be considered when designing treatment. One key informant described a treatment model that very closely matched the ideas focus participants put forth. Another key informant said, “other street-involved people actually look down on solvent users. They’re discriminated against by everyone, so if you throw them in a treatment program that wasn’t designed for them, that doesn’t look at their unique needs, they’re kind of doomed to failure.” One key informant pointed out that many solvent users have major traumas in their life, and asked, “how do you get someone who uses something as a self-medication tool to deal with the trauma sober?” Other issues to consider, pointed out by key informants, included the prevalence of poly-substance use and other addiction issues, the absence of rigorous evaluations of existing solvent-specific treatment elsewhere, and relapse and temptations around friends given the tight knit nature of social networks and lack of additional supports post-treatment.

Focus group participants talked about their concerns regarding on-reserve solvent use. One woman said, “We have a new generation coming out now that’s starting to use the stuff,” and described kids in the age range between 3 and 5 sniffing gasoline and “other stronger stuff.” One key informant said, “Solvent use is happening at a pretty young age and I think they’re continuing this trend when they eventually move to Winnipeg.” This key informant spoke further to the transition into Winnipeg, and said, “On the streets of Winnipeg its almost too late, unfortunately, because they get up in these circles and cycles.” The key informant then mused about solutions, and said, “We’ve got to start working with these young people in communities because a lot of them admit to using in their communities, and when they hit Winnipeg it becomes a lot easier to just connect with that group of individuals.” Echoing that sentiment, one woman said she thought that kids on reserve could use more support. One participant suggested a “1 800 Solvents” phone number, and speaking to communities up North was also suggested.

Generally, the ideas that participants and key informants expressed involved addressing gaps in knowledge and getting solvent use on the radar of society and service providers. Several key informants said that solvent research should be a priority and pointed out several gaps in knowledge that research could address. For example, the long-term effects of chronic and casual solvent use are unknown and the primary harms of solvent use have not been established. Additionally, one key informant said that a better definition of solvent use is needed, with distinction between chronic, intermittent, and

experimental use, and clarity around trajectories and chemical types of solvent use. The addictive nature of solvents has not been investigated, and one participant said, “Some counselors in the city, they figure it’s not a problem, but it is a problem. People are getting addicted to it now.”

3.5: Summary

This study aimed to characterize who solvent users are and what solvent use is in Winnipeg, Manitoba. We completed focus groups with solvent users and individual interviews with solvent users and key informants. We have demonstrated that there is support within the community to work with solvent users and study solvent use. This early phase of research has provided a foundation to build a second phase of research involving the collection of biomedical samples to thoroughly investigate the impact of solvent use on the immune system and HIV disease.

Chapter 4: HLA-B*35 Allele Subtype Frequencies

4.1: Rationale

An epidemiological study, from Mackenzie et al, of all patients with HIV new to care in the Manitoba HIV Program in 2010 showed that individuals of Aboriginal ethnicity were overrepresented and also presenting to care with significantly higher disease burden than Caucasian patients. The study also showed that nearly one quarter of the Aboriginal patients possessed at least one HLA B*35 allele, compared to approximately 14% of Caucasian patients. Additionally, there are low rates of protective alleles (HLA B27 and B57) and high rates of homozygocity, which are associated with rapid disease progression, within the Aboriginal cohort (5). The allele subtype frequency of HLA-B*35 in Manitoba is unknown. Since HLA-B*35 can be divided into two distinct allele subtype groups with different disease progression patterns, it is important to determine which subtypes associated with rapid progression are present, and at what frequencies, within the Manitoba cohort.

4.2: Hypothesis

The HLA-B*35 patient population enrolled in the Manitoba HIV Program will be enriched with HLA B*35 allele subtypes that associate with rapid progression

4.3: Objectives

1. Genotype existing DNA samples using sequence based methods
2. Complete a subset analysis (n=5) correlating high resolution HLA typing results with clinical data regarding disease progression (CD4+ T cell counts and viral load over time, opportunistic infections) and ethnicity

4.4: Results

4.4.1: Study population

Cryo-preserved purified DNA samples from 172 HLA-B35 HIV+ patients from clinics in Manitoba were obtained from Canadian Blood Services (CBS). Between 2007 and 2010, whole blood samples had been collected from HIV+ patients and were received at CBS within seven days of collection. Low resolution typing of the HLA-B locus had previously been completed at CBS using a Lab Type SSO B Locus kit. A non-nominal data set was created by CBS by the removal of identifying information.

4.4.2: Sequence Specific Primer Typing Results

Of the original 172 samples, a subset of the 47 most recent samples (collected between 2008 and 2010) were typed using commercial HLA-B*35 sequence specific primer (SSP) kits from OneLambda. See **Appendix G** for SSP results.

The SSP kits involved a PCR step and a gel electrophoresis step. Representative gel electrophoresis images are shown in **Figure 4.1**. Generally, the kits did not resolve to a

specific allele, except in one case (P08-0111 clearly resolved to B*35:08). There were several reasons for kits not yielding results. There were three cases where the banding pattern was input into the analysis software and the output result was “No allele matches” (**Figure 4.1 D**). Most kits had at least one well with a lack of amplification (**Figure 4.1 C**). The analysis software allowed for non-amplification wells to be marked and for analysis to be done both with and without marked wells.

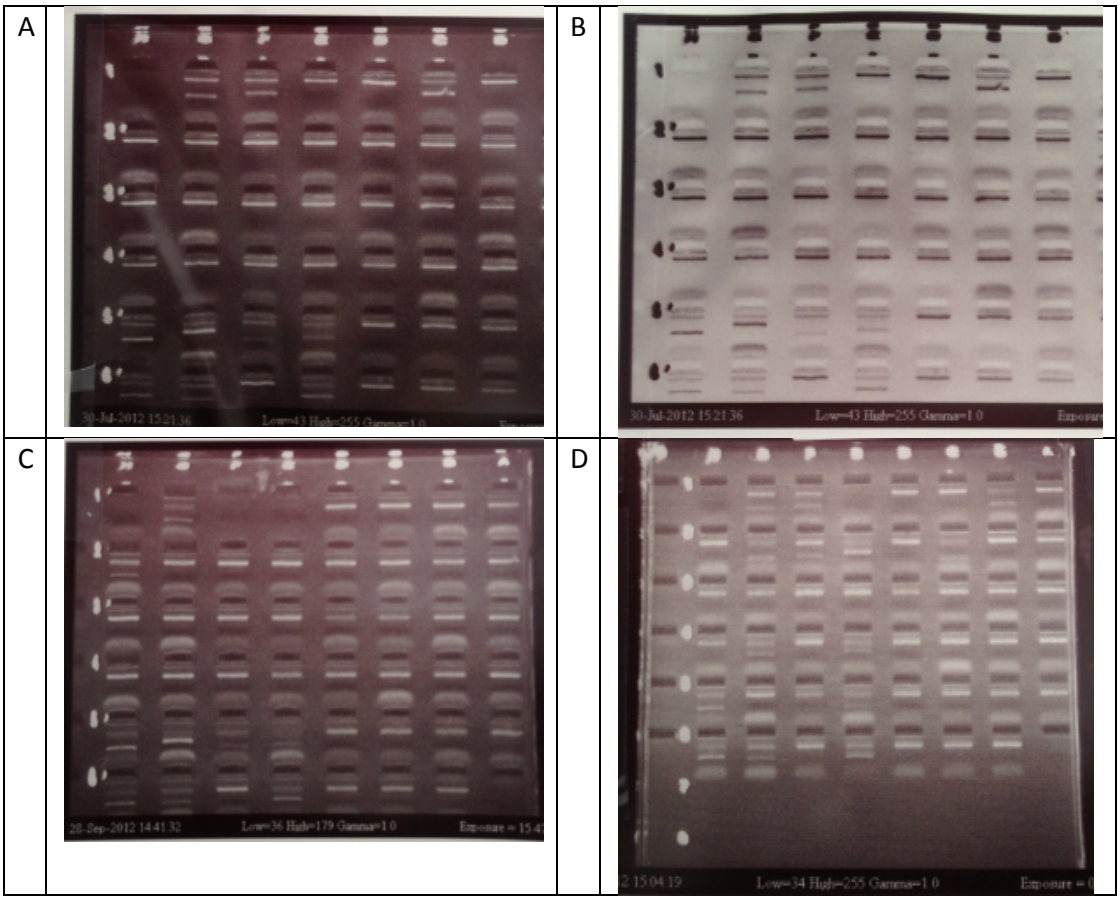

Figure 4.1: Representative gel electrophoresis images from SSP kit protocol. A) and B) are representative of kits that were considered to work and show P08-1507 results with regular and black and white inverted images. C) is representative of a result with non-amplification wells (1E and 1F) but otherwise clear results and shows P08-0163. Pane D) is an example of a kit that did not yield results because the analysis software output was "no allele matches."

4.4.3: Sequence Specific Oligonucleotide Typing Results

Following SSP typing, aliquots of the same 47 samples and gel electrophoresis images were sent to One Lambda for confirmatory HLA-B*35 sequence specific oligonucleotide (SSO) typing. The SSO kits often narrowed down the SSP typing results, but often the results were as ambiguous as the SSP typing (**Appendix H**) Only P08-0111 once again resolving to a specific allele (B*35:08).

4.4.4: HLA Sequencing Results

A total of 167 samples (125 samples from 2007-2008 and 42 of the samples from 2008-2010 that were SSP and SSO typed which had sufficient aliquots DNA left for direct sequencing) were sent for direct sequencing of the HLA-B locus. Of the 167 samples (**Appendix I**) that were sequenced directly, 8 were excluded for the following reasons. Four were excluded because the sequencing protocol did not yield a clear result, and four were excluded because it was discovered they were not, in fact, HLA-B*35. Samples which resolved to have an HLA-B*53:01 allele were also included in this analysis, consistent with other studies (50, 54). A precise high resolution HLA-B*35 or B*53:01 typing result was obtained for 159 samples. For the samples that underwent all three types of typing, results are compared in **Appendix J**.

4.4.5: HLA-B*35 Allele Frequencies

Three methods of sequence-based HLA typing were used to obtain high-resolution HLA-B*35 typing results. Only those samples that resolved to a precise B*35 allele subtype or

B*53:01 through direct sequencing were included in the analysis. Generally, the SSP and SSO kits did not resolve to a specific allele subtype, and as such, only the direct sequencing results were used for analysis.

We identified the presence of eleven HLA-B*35 allele subtypes and the distribution of these HLA-B*35 allele subtypes is presented in **Table 4.1**. The most common subtypes were B*35:01:01G (count = 129, 81.1% of samples) B*35:03:01G (count = 17, 10.7% of samples), B*35:08:01 (count = 5, 3.1% of samples) and B*35:02:01 (count = 3, 1.9% of samples). The allele subtypes B*35:05:01 and B*35:43:01G were both present with counts of 2 and present in 1.3% of samples, and the subtypes B*35:12:01, B*35:20:01, B*35:30, B*35:34, B*53:01 were present at counts of 1 and present in 0.6% of samples.

Regarding the G suffix on B*35:01:01:G, B*35:03:01G, and B*35:43:01G, the G denotes that there are other allele variations that may be correct, but that the allele variations involve polymorphisms that do not occur in exon 2 or 3. A summary of possible alleles subtypes can be found in **Figure 4.2**.

Homozygosity for HLA-B*35 was present in a total of 15 samples (9.4% of samples) (**Table 4.3**). Eleven of these individuals were homozygous at the allele subtype level with homozygosity for B*35:01:01G and B*35:34 present in 6.3% and 0.6% of the samples, respectively. The remaining four individuals, while homozygous for HLA-B*35, were found to be heterozygous at the subtype level.

Of the 159 total samples, 144 were heterozygous and had one HLA-B*35 allele as well as one other different HLA-B allele. Among individuals with one B*35:01:01G allele, the most common second alleles were B*15:01:01G (count = 16, 10.1% of samples), B*40.02:01G (count = 14, 8.8% of samples), B*27:05:02G (count = 12, 7.5% of samples), B*51:01:01G (count = 11, 6.9% of samples), and B*07:02:01G (count = 10, 6.3% of samples). A list of frequencies of all heterozygote pairs present is found in **Table 4.4**.

Table 4.1: Summary of Distribution of the 11 HLA-B*35 allele subtypes in HIV+ patients from Manitoba (n=159)		
Allele	***Count***	***% of population***
B*35:01:01G	129	81.1
B*35:03:01G	17	10.7
B*35:08:01	5	3.1
B*35:02:01	3	1.9
B*35:05:01	2	1.3
B*35:43:01G	2	1.3
B*35:12:01	1	0.6
B*35:20:01	1	0.6
B*35:30	1	0.6
B*35:34	1	0.6
B*53:01	1	0.6

*Note that count column adds to 163 because four individuals, who were homozygous for HLA-B*35 with low resolution typing, were in fact heterozygous for HLA-B*35 at the allele subtype level

Table 4.2: HLA-B*35 alleles with a G suffix, the location of the polymorphism and the possible alleles		
Allele	**Location of Difference**	**Allele List**
B*35:01:01G	None	35:01:01:01
	Exon 1	35:01:23, 35:42:01
	Exon 4	35:01:03, 35:01:25, 35:01:28, 35:40N, 35:57, 35:94, 35:134N, 35:161
	3’ UTR	35:01:01:02
B*35:03:01G	None	35:03:01
	Exon 4	35:70
B*35:43:01G	None	35:43:01
	Exon 4	35:67, 35:79

Table 4.3: Diversity of HLA-B*35 allele subtypes among 15 HIV+ patients from Manitoba who are homozygous for HLA-B*35			
Allele 1	**Allele 2**	***Count***	***% of population (n= 159)***
B*35:01:01G	**B*35:01:01G**	10	6.3
B*35:34	**B*35:34**	1	0.6
B*35:01:01G	**B*35:02:01**	1	0.6
B*35:01:01G	**B*35:03:01G**	1	0.6
B*35:08:01	**B*35:43:01**	1	0.6
B*35:01:01G	**B*53:01**	1	0.6

Table 4.4: Diversity and distribution of HLA-B*35 allele subtypes among 144 HIV+ patients from Manitoba who are heterozygous for HLA-B*35 and one other HLA-B allele			
Allele 1	**Allele 2**	***Count***	***% of total population (n=159)***
B35:01:01G	15:01:01G	16	10.1
	40:02:01G	14	8.8
	27:05:02G	12	7.5
	51:01:01G	11	6.9
	07:02:01G	10	6.3
	44:03:01G	8	5.0
	08:01:01G	8	5.0
	39:01:01G	7	4.4
	40:01:01G	6	3.8
	18:01:01G	4	2.5
	48:01:01G	3	1.9
	14:02:01	3	1.9
	47:03	2	1.3
	41:02:01	2	1.3
	57:01:01G	2	1.3
	45:01G	1	0.6
	48:07	1	0.6
	50:01:01	1	0.6
	38:01:01G	1	0.6
	58:02	1	0.6
	57:03:01	1	0.6
	38:02:01G	1	0.6
	13:02:01G	1	0.6
B35:03:01G	07:02:01G	2	1.3
	51:01:01G	2	1.3
	18:01:01G	2	1.3
	27:05:02G	2	1.3
	15:01:01G	2	1.3
	39:06:02G	1	0.6
	13:02:01G	1	0.6
	52:01:01G	1	0.6
	14:02:01	1	0.6
	39:10:01	1	0.6
	08:01:01G	1	0.6
B35:08:01	07:02:01G	1	0.6
	39:01:01G	1	0.6
	40:02:01G	1	0.6
	08:01:01G	1	0.6
B35:02:01	08:01:01G	1	0.6

	51:01:01G	1	0.6
B35:05:01	08:01:01G	1	0.6
	15:13:01	1	0.6
B35:43:01G	14:01:01	1	0.6
B35:12:01	15:01:01G	1	0.6
B35:20:01	15:01:01G	1	0.6
B35:30	15:35	1	0.6

4.4.6: Case Study on HLA-B*35 samples from 2010

Clinical information was obtained from five patients from Manitoba through a review of medical charts. Two of the five patients were deceased and limited information was available. For the remaining three patients, information on $CD4^+$ T cell counts and viral load were available, as well as other relevant clinical and personal data since their diagnosis with HIV.

Individual P10-1236

Genotype: B*35:01:01G / B*15:01:01G

Individual P10-1236 was an Aboriginal male that was diagnosed with HIV in 2010. He died in June 2011 due to a respiratory illness.

Individual P10-2075

Genotype: B*35:01:01G / B*35:01:01G

Individual P10-1236 was an Aboriginal female that was diagnosed with HIV in 2010. She died in July 2011 due to liver failure.

Individual P10-1188

Genotype: B*35:01:01G / B*44:03:01G

Individual P10-1188 was a Caucasian female that was diagnosed with HIV in April 2002. Upon diagnosis, she had a $CD4^+$ T cell count of 470 cells/μL and a viral load of 1800 copies/mL. Her risk factors for HIV acquisition were unprotected sex with a male, sex

with someone with known HIV, intravenous drug use, tattoos, and involvement in the sex trade. Clinical notes reported that the woman experienced issues with adherence to ARVs. Indeed, she was unable to consistently keep viral loads at undetectable levels throughout the course of disease and also experienced modest improvements in $CD4^+$ T cell count (**Figure 4.2**).

Regarding other medical history, the woman had a case of bacterial vaginosis in April 2002, and a history of recurrent urinary tract infections. She tested positive for cytomegalovirus (CMV) IgG in 2002 and positive for Hepatitis A antibodies (HAV-Ab) in 2010. Some information regarding social factors and mental health were obtained from the medical charts. The patient had two children who were both adopted out. She had a history of using crack cocaine and regularly using marijuana. The charts indicated that she was experiencing a lot of "social stressors" and had a history of depression.

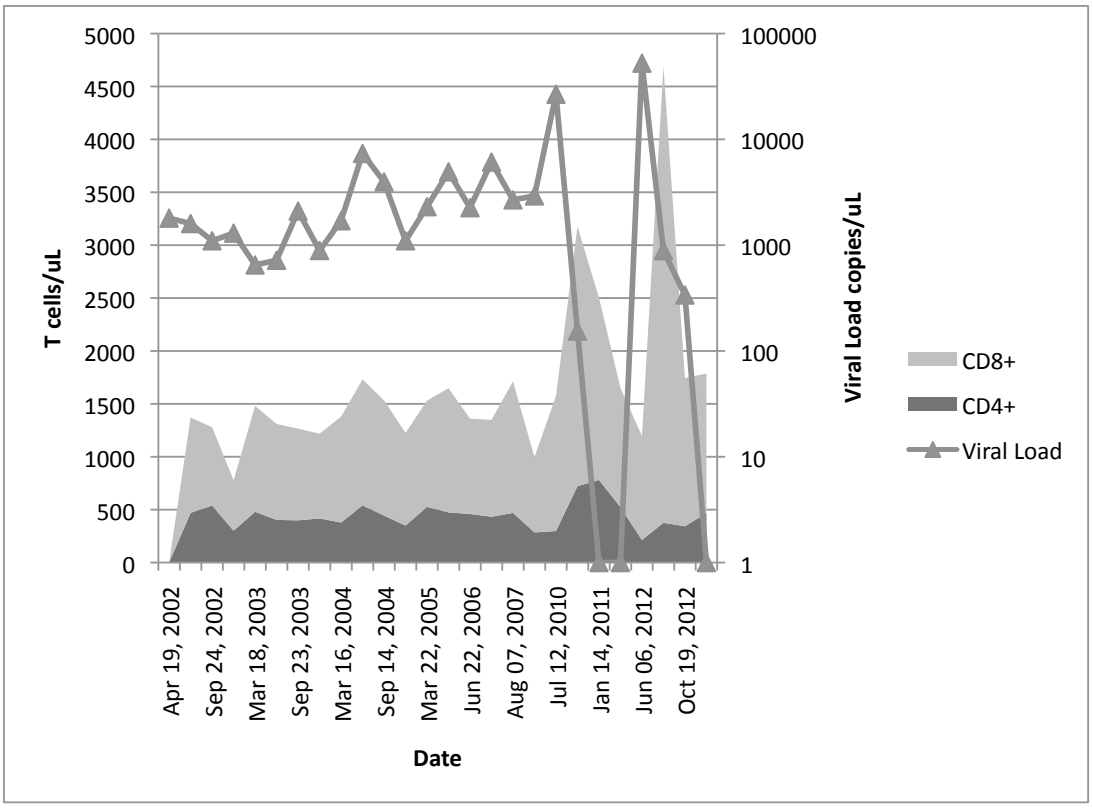

Figure 4.2: HIV disease progression indicators for Individual P10-1188 including $CD4^+$ and $CD8^+$ T cell counts over time from July 2002 until February 2013 and viral load over time from April 2002 until October 2012

Individual P10-1675

B*35:01:01G / B*15:01:01G

Individual P10-1675 was a First Nations woman that was diagnosed with HIV in 2010, at the age of 27, and had an initial $CD4^+$ T cell count of 14 cells/μL and a viral load of >1million copies/mL. She presented to care with two opportunistic infections, oral thrush and *Pneumocystis jiroveci* Pneumonia. Her risk factors for HIV acquisition were unprotected sex with a male, and sex with a person from a high-risk country. She was placed on antiretroviral therapy and $CD4^+$ T cell counts rebounded towards healthy levels while viral load dropped to undetectable (**Figure 4.3**). According to the physician's notes, she is "doing well" and 100% compliant with medication.

Regarding other medical history, she was positive for CMV IgG and HAV-Ab in September of 2010 and reported Herpes and oral thrush in November 2010. The patient reported that although she had a very supportive family, she was battling ongoing stress, subject to depression, and self-described anxiety. There was a previous history of weekend alcohol binging and she reported that she had previously tried marijuana.

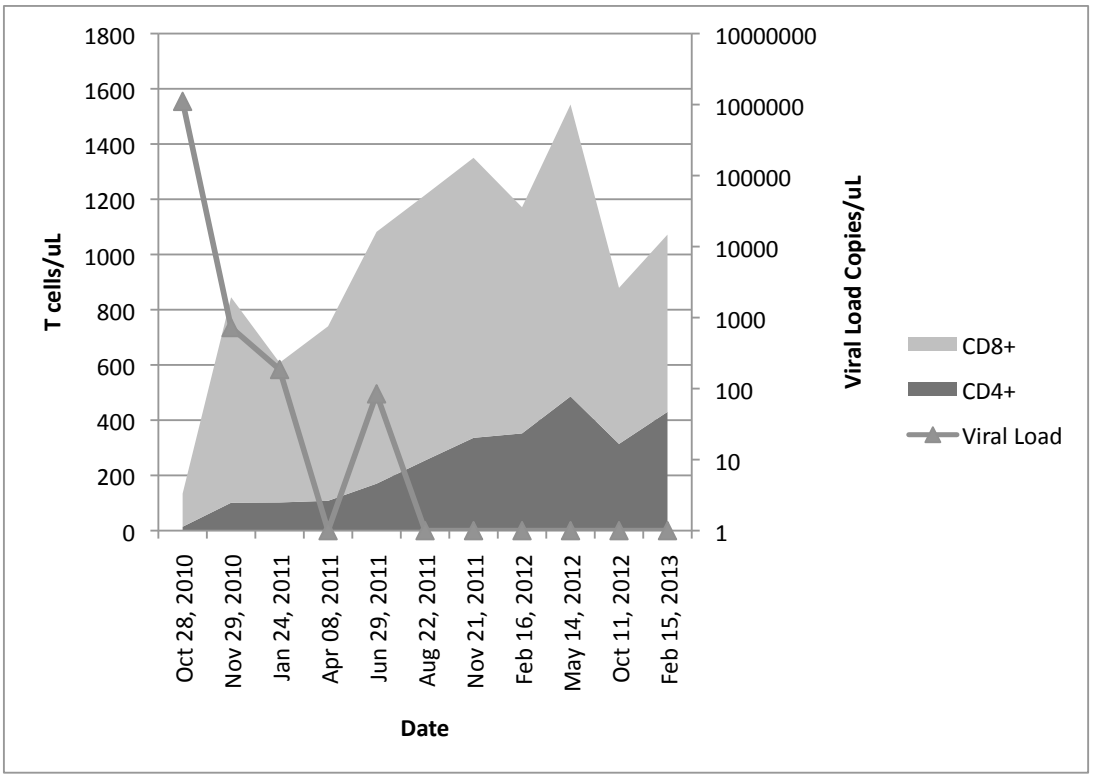

Figure 4.3: HIV disease progression indicators for Individual P10-1675 including $CD4^+$ and $CD8^+$ T cell counts over time from October 2010 until February 2013 and viral load over time from October 2010 until February 2013

Individual P10-0137

B*35:01:01G / B*35:01:01G

Individual P10-0137 was a Métis male who was diagnosed with HIV in January 2010, at the age of 27. Upon presentation to care, his initial $CD4^+$ T cell count was 14 cells/μL and initial viral load was 58 900 copies/mL. He did not have any opportunistic infections. His risk factors for HIV acquisition were unprotected sex with a female, and sexual assault. The HIV physician reported issues with antiretroviral compliance and $CD4^+$ T cell counts did not rebound beyond 157 cells/μL while viral load remained detectable and regularly over 80 000 copies/mL (**Figure 4.4**). Indeed, the HIV physician noted that, "It seems [the patient] has had rapid progression with respect to HIV"

Regarding other medical history, the patient tested positive for CMV IgG in February 2010 and presented with "pretty bad" oral thrush in March 2010. There was also a history of poor mental health status and difficulty coping with the diagnosis. The patient was receiving HIV-specific medical care in Winnipeg and living in Northern Manitoba. The correspondences between the HIV physician and family doctor provided information regarding social issues. The patient has a history of incarceration and also reported a history of smoking crack cocaine, drinking alcohol, and smoking marijuana. He continued to smoke marijuana for symptom control of HIV. The HIV physician's notes reported that the patient appeared to be struggling with social situations, and was being discriminated against in his home community because of his HIV status.

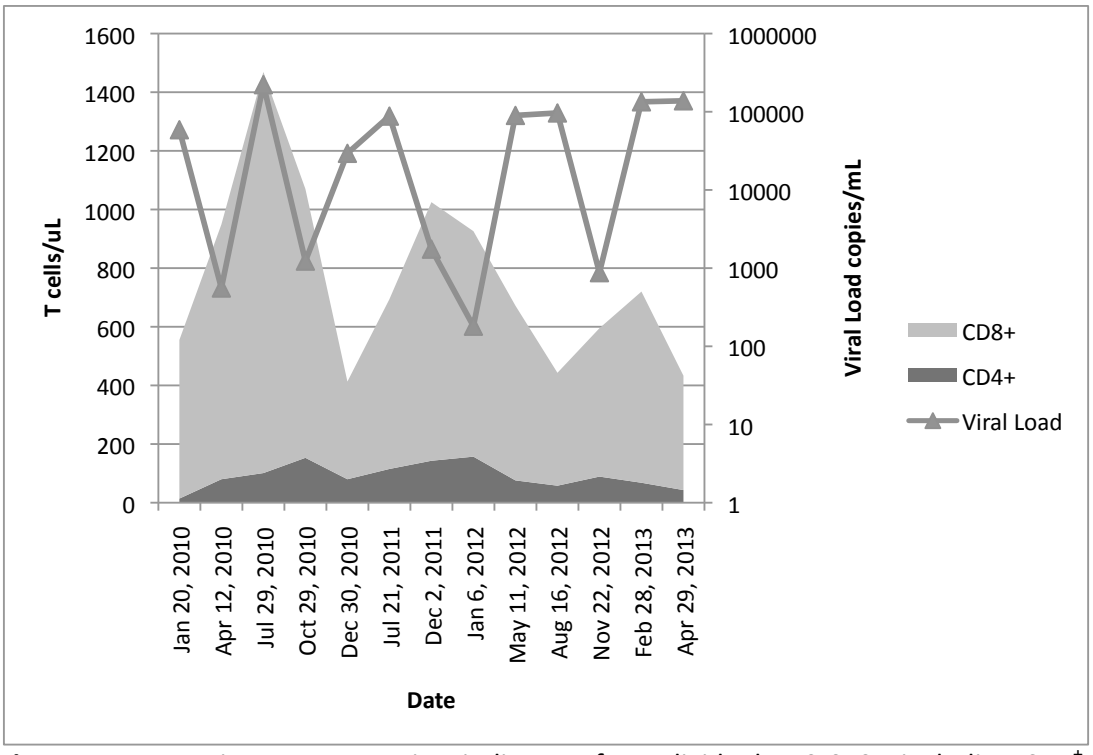

Figure 4.4: HIV Disease progression indicators for Individual P10-0137 including $CD4^+$ and $CD8^+$ T cell counts over time from January 2010 until April 2013 and viral load over time from January 2010 until April 2013

4.5: Summary

This study aimed to identify the diversity and frequency of HLA-B*35 allele subtypes, at the high resolution level, in the HIV+ population in Manitoba that presented to care between 2007 and 2010. We observed that 11 distinct HLA-B*35 allele subtypes exist within the population and that the most common allele subtypes are B*35:01:01G, B*35:03:01G, and B*35:08:01.

We did case studies on patients from 2010 where clinical chart information was available. Although the sample size was small for this subset analysis, the patient records reflected the overrepresentation of Aboriginal people infected with HIV in Manitoba. The pressing issues of late presentation to care and rapid disease progression also appeared to be present, although there was insufficient chart information available to clearly place individuals into one of these categories.

Chapter 5: Discussion

Manitoba's HIV epidemic is characterized by both an overrepresentation of Aboriginal peoples and an abundance of individuals, especially Aboriginal peoples, presenting late to care or with advanced HIV disease. The Manitoba HIV Program, in their 2011 Program Update Report, stated that an effective response in Manitoba called for both multiple contributing voices and an understanding of contextual factors (4). This thesis aimed to answer that call through an interdisciplinary investigation exploring host factors relevant to the HIV epidemic in Manitoba. Two distinct projects were undertaken. The first project explored solvent use with a community-based research approach, and the second project explored HLA-B*35 allele subtype diversity using a basic science approach.

5.1: Solvent Project

This project aimed to investigate the link between solvent use and HIV through a multi-phase interdisciplinary research project. The work presented in this thesis represents the first phase of this research and sought to answer four main research questions:

1. What is solvent use in the context of Winnipeg, Manitoba?
2. Who are solvent users in the context of Winnipeg, Manitoba?
3. To what extent is the research team and approach accepted by the solvent-using population?

4. To what extent is a second phase of project, involving biomedical research, feasible?

The richness and depth of information provided to the study team was captured by focus groups and qualitative interviews and could not have been gathered by a quantitative study alone. The results of this phase of the study have informed the next basic-science driven phase aimed at investigating biological and immunological links between solvent use and HIV. More importantly, perhaps, the findings of the first phase lent a voice to one of the most vulnerable and stigmatized groups of people in Manitoba. The results provided insight into the lived experiences of individuals who use solvents and individuals who work closely with this population. In addition to discussing the findings related to the main research questions, this section will discuss unmet needs of the population that were part of the study and current gaps in knowledge and health services. Together, these factors illuminate where there are disparities regarding the social determinants of health and therefore present opportunities for meaningful engagement between the solvent using population and interdisciplinary teams.

5.1.1: Addressing Research Questions

Given that the vast majority of solvent use research focuses on youth (20-22, 24, 25), Manitoba appears to have a demographically unique population of solvent users, in terms of older age and patterns of use. At the onset of the first phase of the study, we wondered if the project would be feasible at the community level. Specifically, we wondered if the research team would be accepted, if there would be interest from the

solvent using population to participate, and if a second phase of research involving blood sample acquisition would be possible. To address these issues we took the approach of conducting nine qualitative interviews and three focus groups with solvent users as well as six interviews with key informants. Key informants included individuals who identified as service providers, physicians, and researchers.

The solvent using population demonstrated a keen interest and willingness to meaningfully engage with the research team and participate in the study, including an interest in the donation of blood for biological analyses for later phases. During the preliminary individual interviews with solvent users, we asked questions relevant to mucosal (gastrointestinal and respiratory) health. Stomachaches and vomiting were often associated with heavy use of solvents. Some participants also noted coughing and spitting up phlegm, and others described changes to their skin such as sweating out a gummy substance or getting white patches. We believe that these observations merit further investigation into the impact of solvent use on gastrointestinal, respiratory, and dermatological health.

5.1.2: Unmet Needs of the Population/Identified Gaps in Knowledge

5.1.2.1: Access to Primary Care

Based on key informant interviews, there appears to be two major barriers to solvent users accessing primary health care. The first is that some local clinics have a “no sniffing” policy and deny services to solvent users, and the second barrier is that

appointment-based primary care may not be the best approach. Several key informants said that a major barrier to health service delivery is the long-term nature of health and wellness programs versus the day-to-day survival of individuals who use solvents. It may be that this often translates into health service providers and policy makers assuming that solvent users do not care about their personal health and well-being. Interviews with solvent users demonstrated that, while some participants did indeed have little regard for their health and expressed hopelessness, many we spoke with were committed to attending frequent visits with physicians for chronic health issues as well as sexually transmitted blood borne infections (STBBI) testing.

5.1.2.2: Access to Hepatitis C and HIV Medications

We did not specifically ask participants to disclose HIV or Hepatitis C status. While none of the solvent users we interviewed voluntarily disclosed HIV status, some did disclose that they were Hepatitis C positive. Additionally, key informants that work with the population discussed the presence of HIV within the population. Structural challenges when treating drug users with HCV have previously been identified and include poverty, homelessness, mental health issues including addictions, and issues around successful and trusting physician-patient relationships (79). These factors were also identified as issues in our study. Key informants spoke to the difficulty of treatment and management of infectious diseases like HIV and HCV among those who are concurrently using drugs. Indeed, one participant stated that their physician informed them that staying “clean” was a necessary step before starting treatment. Whether this is standard

clinical procedure warrants further investigation given that a robust body of literature supports the conclusion that physicians should not withhold treatment for HCV based on the presence of a concurrent substance use addiction (79-81).

When it comes to HIV medications, adherence is important given that poor adherence predicts poor clinical outcome due to the development of resistance. It has been demonstrated that physicians are often incorrect when deciding if patients will be adherent, but that some behaviors, such as missing appointments (82) and a history of alcoholism or intravenous drug use (83), can be predictive of poor adherence (82). Given that key informants noted poor attendance for appointment-based primary care and poly-substance users, it was not surprising that key informants noted that ARV compliance is a challenge.

Key informants also had suggestions for other ways to improve adherence. One key informant heralded the benefits of including incentive medications, like Tylenol 3s and antidepressants, in bubble packs with HIV medications. This approach is problematic on multiple levels. First, the chemical interactions between solvents, other drugs that individuals may be using, and prescription drugs are largely unknown, and could in fact be dangerous. Second, Tylenol 3 contains codeine, which is an addictive opiate (84). Non-medical opiate use has become a major public health challenge in Canada and appears to be fueled by over-dispensing (85). Certainly, dispensing Tylenol 3s as an incentive for taking medications is highly questionable, especially when considering that

many solvent users we spoke to had histories of substance use and dependency. This practice warrants further investigation. The success of a pharmaceutical-based incentive program suggests that there is an opportunity to develop a better, less dangerous, incentive program. For example, cash-based incentive programs may be an effective alternate avenue to pursue (86) as discussed by Pettifor et al, who suggest that cash-incentive programs may be effective, depending on context and purpose of the program as well as the population (86).

5.1.2.3: Health Services for Pregnant Women

The study identified a major gap in health services for pregnant women who are solvent users. Several female participants described using solvents while pregnant, and several male participants talked about pregnant women in their lives using solvents. Considering that solvent use is an appetite suppressor, the nutritional hazards that solvent use could have on fetal development are worrisome. Additionally a key informant spoke about a local organization that provides services for mothers at risk for fetal alcohol syndrome turning away mothers who are solvent users. Clearly, there needs to be more support and resources for pregnant women who are using solvents.

5.1.2.4: Mental Health Services

Many participants and key informants discussed significant traumatic events in the lives of solvent users and described solvent use as a coping mechanism. Additionally, a few participants talked about solvent users committing suicide, or they themselves having

suicidal thoughts. Mental health support appeared to be sought through close friends, older relatives, elders, and religious friends. Participants reflected on encounters with psychologists and psychiatrists negatively. Given the preponderance of solvents as a coping mechanism, culturally sensitive and respectful mental health services could be beneficial. We asked participants what kind of mental health support would be best in the context of future research, and the majority of participants said a person who had lived similar experiences, or was willing to learn about their lives without being judgmental, would be best.

5.1.2.5: Infectious Disease Prevention Tools and Education

Key informants talked about solvent users who also use intravenous drugs. One person we interviewed was currently injecting Talwin and Ritalin, and four others recalled instances of IDU from their past. Most participants knew to avoid sharing needles, but it appeared as though other IDU tools were shared, like water, spoons and filters. Additionally, most participants had a history of smoking crack cocaine, however, availability of safe crack kits were not discussed with participants. Although policies around the distribution of harm reduction supplies, including clean needles and safe crack kits, remains controversial in Canada, the effectiveness of these tools in preventing virus transmission is clear (87, 88). The Winnipeg Regional Health Authority's Street Connections van is involved in distributing safe crack kits and clean needles to drug users and organizations that provide services to drug users (89). One key informant

mentioned that a local shelter destroyed client's harm reduction supplies. This claim warrants further investigation.

A dichotomy was revealed regarding sexual risk for STI transmission. Key informants indicated that they perceived solvent users were at a heightened risk for sexual transmission of HIV due to neglecting to use condoms and a tendency for more promiscuous behavior. Conversely, solvent users perceived that solvents made them less desiring of sex and did not negatively impact their likelihood of using condoms. This clear discrepancy in potential risk warrants further investigation to better understand the relationship between solvent use and sexual risk.

5.1.2.6: Addictions Treatment

The lack of treatment facilities for adult solvent users emerged at the forefront of gaps in services for solvent users. Furthermore, there was evidence of a lack of general knowledge about solvent use addictions and effects on the body. Both key informants and participants conveyed the need for solvent-specific treatment centre in Winnipeg. Additionally, both key informants and participants had many ideas for what a successful treatment centre could be like. It appears as though, in Winnipeg, there is both the need and interest to develop such a facility.

5.2: HLA-B*35 Project

Host genetic factors can influence HIV disease susceptibility and disease progression. HLA-B*35 is one such genetic factor that has been shown to be associated with rapid disease progression in other cohorts around the world (65). To determine the diversity of HLA-B*35 allele subtypes in Manitoba, we used high-resolution sequence based methods to find the subtypes of 159 individuals from Manitoba who presented to care between 2007 and 2010. The hypothesis for this project was: The HLA-B*35 patient population enrolled in the Manitoba HIV program will be enriched with HLA-B*35 allele subtypes that associate with rapid progression.

5.2.1: Diversity and Distribution of HLA-B*35 Allele Subtypes Sssociated with Disease Progression

We identified 11 distinct HLA-B*35 allele subtypes, which can be divided into two categories: First, those which are known to associate with HIV rapid disease progression (35:03, 35:02, 53:01) or thought to associate with rapid progression due to their peptide binding specificities (35:12 and 35:34) (41). Second, those which are known to associate with HIV regular disease progression (35:01, 35:08), and those which are suggested to be associated with regular disease progression based on peptide binding specificities (35:05, 35:43, 35:30, and 35:20) (41). Below is a discussion of each allele subtype with consideration of the percentage of the study population that had each allele subtype. The allelefrequency.net database (56) was used to identify percentage ranges of allele subtypes from general populations around the world. These ranges are summarized in

Table 5.1. Please not that these general populations were not specifically cohorts of HIV+ individuals, and that information about HLA-B*35 subtype ranges in HIV infected populations for the identified allele subtypes was not available.

Four PX allele subtypes (35:03, 35:02, 35:12, 35:34 and 53:01) associated with rapid disease progression were found in the Manitoba population. Allele subtype 35:03 was present in 10.7% of the study samples, making it the second most frequent subtype. This allele has been found to range between 0.1% (Taiwan) and 10.6% (India), when present, in general populations (56). Allele subtype 35:02, was present in 1.9% of the study samples. This allele has been found in general populations to range between 3% (England) and 18% (USA) (56). The allele subtypes 35:12, 35:34, and 53:01 were each in 0.6% of study samples. The allele subtype 35:12 has been found ranging between 0.1% (Eastern Europe) and 24% (Mexico) (56), and was first identified in a Brazilian Indian tribe (90). The allele subtype 35:34 has been found ranging form 3% (Kenya) to 5% (Portugal) (56). The allele subtype 53:01 has been found to range from 0.1% (Poland) and 24% (São Tomé Island) (56). Considering the number of records for each allele subtype on the allelefrequency.net database, it appears as though the most common alleles are 53:01, 35:03 and 35:02 (which have 163, 144, and 109 records, respectively). Relative to these record counts, 35:12 and 35:34 have rarely been found in world populations, with record counts of 69 and 14, respectively. Thus, it appears as though our study cohort has rare allele subtypes that are associated with rapid disease progression.

Six PY subtypes (35:01, 35:08, 35:05, 35:43, 35:30, and 35:20), associated with regular progression, were found in the study samples. Allele subtype 35:01 was the most frequent allele subtype and present in 81.1% of samples. HLA-B*35:01 is the most common allele subtype in the world and is universally distributed (55). When present in a population, its frequency ranges from between 3% (Indonesia) and 48.5% (Mexico) in general populations. It has been found to be present at frequencies of approximately 20% in American Indian populations (56). Allele subtype 35:08 was the third most frequent allele subtype and found in 3.1% of samples. This allele subtype has been found to range from 2% (China) to 4.5% (Bulgaria) in general populations where it is present (56). The allele subtypes 35:05 and 35:43 were both present 1.3% of samples. Allele subtype 35:05 has been found in populations ranging from 0.1% (Germany) and 51% (Peru) (56) and was first identified from a Brazilian Indian tribe (90). The allele subtype 35:43 has been found in populations ranging from 0.4% (USA) and 1% (Mexico). This allele was identified in the mid 1990s in both a Bari tribe from Venezuala (91) and a Cayapa tribe from South America (92). The allele subtypes, 35:20, 35:30, and were each found in 0.6% of study samples. The allele subtype 35:20 has been found ranging from 1.5% (USA Hispanic) and 1.2% (Singapore) (56), and was first found in a tribe of South American Indians (93). The allele subtype 35:30 has been found ranging between 0.1% (USA Hispanic) to 0.3% (Indonesia) when present (56).

Table 5.1: Summary of 11 HLA-B*35 allele subtypes found in patients enrolled into the Manitoba HIV Program from 2007-2010 including information regarding association with HIV disease progression, their distribution percentage within the study samples, and their distribution percentage range in general world populations when present

Association with HIV disease progression	Allele Subtype	% of Study Sample Population (n = 159)	% Range in General World Populations (when present)
Rapid disease progression (PX subtype)	35:03	10.7	0.1 (Taiwan) – 10.6 (India)
	35:02	1.9	0.3 (England) – 18.0 (USA)
	53:01	0.6	0.1 (Poland) – 24.4 (São Tomé Island)
	35:12	0.6	0.1 (Eastern Europe) – 24.0 (Mexico)
	35:34	0.6	0.3 (Kenya) – 0.5 (Portugal)
Regular disease progression (PY subtype)	35:01	81.1	0.3 (Indonesia) – 48.5 (Mexico)
	35:08	3.1	0.2 (China) – 4.5 (Bulgaria)
	35:05	1.3	0.01 (Germany) – 51.0 (Peru)
	35:43	1.3	0.04 (USA) – 1.0 (Mexico)
	35:30	0.6	0.1 (USA Hispanic) - 0.3 (Indonesia)
	35:20	0.6	1.5 (USA Hispanic) – 1.2 (Singapore)

* Please note that column three shows percentages of each B*35 allele subtype from the B*35 containing Manitoba HIV+ population. The percentages in column four represent populations from around the world, not just HIV+ people or people with one B*35 allele.

Diversity of B*35 subtypes has been observed at different frequencies in other populations. For example, a recent study from Croatia identified the presence of a total of four distinct B*35 allele subtypes (55) and an Italian study of several thousand participants observed a total of thirteen distinct B*35 allele subtypes, most of which occurred at frequencies of less than 1% (57). Indeed, with 11 allele subtypes present, it appears as though the Manitoba HIV positive population is diverse in terms of HLA-B*35 allele subtypes.

This data identifies that, out of 159 study participants, 23 individuals presenting to care in Manitoba between 2007 and 2010 had one HLA-B*35 allele subtypes that is associated with rapid progression. This type of information would be useful for physicians when determining if and when a patient is placed on antiretroviral therapy, and supports the uptake of high resolution typing at the HLA-B*35 locus as standard clinical procedure. The next sections of this discussion considers homozygosity for HLA-B*35 and the presence of other deleterious alleles.

5.2.2: Frequency and Distribution of Homozygous and Heterozygous HLA-B*35 Allele Subtypes Associated with HIV Disease Progression

Preliminary low resolution typing results from Canadian Blood Services identified 15 individuals who were homozogyous for HLA-B35. High resolution typing revealed that 6.3% of the study samples were homozygous for 35:01, and that 0.6% were homozygous for 35:34. The remainder of the individuals identified as being homozygous for B*35

with low resolution typing were found to be heterozygous at the allele subtype level. Homozygosity at B*35 is associated with rapid disease progression regardless of allele subtypes present. Physicians should take HLA-B*35 homozygosity into consideration when identifying if and when a patient be placed on antiretroviral therapy, and this decision should not be contingent on high resolution typing results.

The study cohort consisted of 144 individuals who were heterozygous for HLA-B35. Among this group there existed 29 other HLA-B alleles, several of which have been associated with HIV disease progression. The alleles associated with rapid progression were: 15:01, 07:02 (94), 08:01, 18:01 (95), 45:01, and 58:02 (96). The alleles associated with protection were: 27:05, 14:02 (44), 57:01, 57:03 (96, 97), 13:02 (98), and 39:10 (99).

One allele of particular interest was HLA-B*51 which is typically associated with protection (100). A recent publication from Manitoba noted that, in 2010, 31% of patients that were considered to be late presenters had at least one HLA-B*51 allele. It was speculated that the abundance of this typically protective allele in late presenters may be related to a phenomenon that has occurred in Japan – where over time, B*51 has lost its protective effect (101). Given that our data shows the presence of many other HLA alleles associated with rapid disease progression, it is also possible that that B*51 individuals in Manitoba presenting late to care or with accelerated disease progression are heterozygous with a second deleterious allele.

The **Figure 5.1** illustrates the breakdown of alleles associated with rapid progression, regular progression, and protection as well as alleles with no known association to HIV disease progression and the B*51 allele. Of the 144 individuals who were heterozygous, 19 had a B*35 associated with rapid progression, and 9 of these individuals had a second allele that was also associated with rapid progression. Notably, of the 125 individuals who were heterozygous with a B*35 associated with regular progression, 44 of these individuals had a second non B*35 allele associated with rapid progression.

This data shows that in addition to the 15 individuals who were homozygous for B*35, 19 of the heterozygote individuals had a B*35 that associated with rapid progression. Furthermore, there were 44 individuals who had one B*35 that associated with regular progression and a second allele that associated with rapid progression. Therefore, of the original 159 samples, 78 individuals had at least one allele that is associated with rapid progression. This means that approximately half of the study samples were from individuals who possess a genetic predisposition associated with rapid HIV disease progression. This data supports the hypothesis that the HLA-B*35 patient population in the Manitoba HIV program is enriched with HLA-B*35 allele subtypes associated with rapid progression. The impact of these alleles on disease progression in Manitoba warrants further investigation.

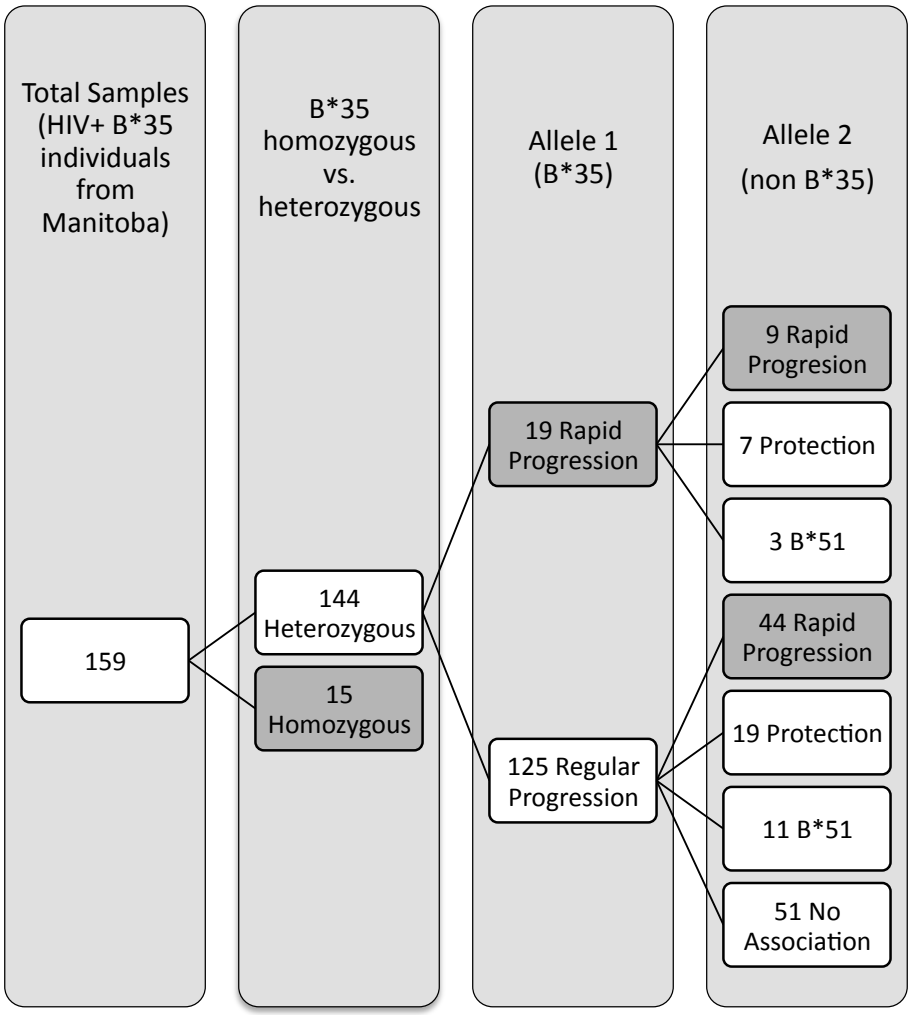

Figure 5.1: Breakdown of alleles in study population (n= 159) associated with rapid progression, regular progression, and protection as well as alleles with no known association to HIV disease progression and the B*51 allele. Shaded boxes denote genotypes associated with rapid progression

5.2.3: Case Reports

Five case reports highlighted some of the important issues that individuals with HIV experience while living in Manitoba and suggest the role that their genetics may play in disease outcomes.

Two individuals, both diagnosed with HIV in 2010, died in 2011 and had limited clinical information available. The first person (P10-1236), an Aboriginal male, had B*35:01, which is associated with regular disease progression, and B*15:01, which is associated with rapid disease progression. This individual passed away due to a respiratory illness. The second individual (P10-2075), was an Aboriginal female and was homozygous for B*35:01, which is associated with rapid disease progression. This individual passed away due to liver failure. Although information about viral load and $CD4^+$ T cell count was not available, the case reports suggest that these two individuals likely presented late in the course of their HIV disease or progressed rapidly, given that they both lived less than a year following initial diagnosis. This data lends support to the disadvantage of B*35 homozygosity and the deleterious effects of other alleles associated with rapid progression.

Clinical information regarding disease progression was available for three individuals.
The first case was a Caucasian female (P10-1188) who presented to care in 2002 with near-normal $CD4^+$ T cell counts and low viral load. Her HLA genotype was found to be B*35:01 and B*44:03, with the latter not clearly associating with a disease progression

pattern. Based on her HLA typing results, she would be expected to not experience rapid disease progression. Indeed, her issues regarding maintaining low viral loads and high CD4+ T cell counts were attributed to problems with ARV adherence. This participant had a history of intravenous drug use and involvement in the sex trade, highlighting the importance of outreach and prevention efforts with sex trade workers and intravenous drug users in Manitoba.

The second individual (P10-1675) was a First Nations woman that presented to care with advanced HIV disease, very high viral loads, and multiple opportunistic infections. Her genotype was B*35:01 and B*15:01, the latter being associated with rapid disease progression. She was placed on ARVs and was reported to be doing well and adhering well to medications. Her clinical notes mentioned a supportive family network, which highlights the importance of social supports while coping with HIV diagnoses.

The last individual was a Métis male that also presented with advanced HIV disease. Genetically, he was at a disadvantage since he was homozygous for B*35. Since his diagnosis, he has struggled to control his HIV disease. Besides genetics, other factors that are likely very important in terms of his welfare are unstable housing, dealing with HIV in rural areas of Manitoba, and community stigma. He also has a history of incarceration.

Together, these case reports illustrate the complex factors that can influence disease progression in Manitoba and highlight the effect host genetics may have on HIV disease progression.

5.3: Contribution to the Field

This thesis addressed and investigated two principal gaps in knowledge regarding HIV disease and risk factors in Manitoba using a unique research approach. The first project addressed a cross-discipline interest into the behaviors and characteristics of adult solvent users in Manitoba, and provided a foundation for ongoing immunological studies with this population. The second, built on previous work from the Manitoba HIV Program, involved the identification of 11 HLA-B*35 allele subtypes within the Manitoba HIV positive population and presented evidence that high resolution typing of B*35 individuals, which is not part of current clinical practice, may provide a way to predict rapid disease progression which could lead to tighter follow up and earlier treatment of patients. The interdisciplinary research approach, in itself, can be considered an experiment in new approaches to tackling problems at the intersection between complex biological and social issues.

5.4: Limitations

There were several limitations to the studies described within this thesis. Regarding the solvent use study, we were limited in sample size, and the voices we heard may not be representative of the broader solvent using population. Participants who signed up to

be a part of the project may have been more interested in personal health than other solvent users, and this may have introduced bias into the results. We also did not recruit any individuals who self-identified as HIV positive. All of the individuals we interviewed were adults and this study thus lacked the important opinions and experiences of youth solvent users. Most of the key informants interviewed had experienced successes in terms of engaging with, and providing programming or services for solvent users and we did not capture the voice of key informants who had experiences of excluding solvent users from programming or services.

The HLA study also had several limitations. First, we were unable to obtain clinical information relevant to disease progression or ethnicity data for patients from 2007-2009, which were the bulk of samples studied. The case study portion was also limited by a small sample size. Lastly, we did not have HLA-B*35 allele subtype frequencies from the general Manitoba population or other similar populations, which would have allowed us to complete statistical analysis.

5.5: Concluding Remarks and Future Directions

This thesis explored both social and biological factors relevant to the HIV epidemic in Manitoba. Two distinct projects were undertaken.

The first project aimed to investigate the link between solvent use and HIV through a multi-phase interdisciplinary research project. The work presented in this thesis

represents the first phase of this research and sought to characterize solvent users and solvent use as well as assess acceptability of the research team, approach, and later phases of research. The results provided insight into the lived experiences of individuals who use solvents and individuals who work closely with this population. In addition, several gaps in knowledge and health services were identified. Together, these factors illuminate where there are disparities regarding the social determinants of health and therefore present opportunities for meaningful engagement between the solvent using population and interdisciplinary teams. The work presented in this thesis creates a foundation for investigating biological factors that may influence HIV risk in solvent using population. Furthermore, solvent use is certainly not unique to Winnipeg. Establishing a research approach in Winnipeg will be helpful in exploring the link between HIV and solvent use locally and internationally.

The second project investigated the host genetic factor, HLA-B*35, which is associated with rapid disease progression. We hypothesized that the HLA-B*35 patient population enrolled in the Manitoba HIV Program would be enriched with HLA-B*35 allele subtypes that are associated with rapid progression. When considering both the allele subtype distribution data, it is clear that allele subtypes associated with HIV rapid disease progression are enriched within this cohort and that these alleles may in fact be exerting an influence. This study lends support to the idea that HLA genotyping HIV positive patients, at the high resolution level, may be a useful tool when trying to determine patient follow up schedules and if a patient should be placed on ARVs.

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Chapter 7: Appendices

Appendix A: List of Abbreviations

AIDS – acquired immunodeficiency syndrome
ARV – antiretroviral
BSC – biological safety cabinet
CBS – Canada Blood Services
CBR – community based research
CD – cluster of differentiation
cDNA – complementary deoxyribonucleic acid
CMV – cytomegalovirus
CTL – cytotoxic T lymphocyte
DNA – deoxyribonucleic acid
ddH_2O – double distilled water
dNTP – deoxyribonucleotide triphosphate
ddNTP – dideoxyribonucleotide triphosphate
EDTA – ethylenediaminetetraacetic acid
EtBr – ethidium bromide
GALT – gastrointestinal associated lymphoid tissue
GI – gastrointestinal
H_2O – water
HAV-Ab – Hepatitis A antibodies
HCl – hydrochloric acid
Hep C – Hepatitis C virus
HIV – human immunodeficiency virus
HLA – human leukocyte antigen
IDU – intravenous drug use
IMGT – The International ImMunoGeneTics information system
LPS – lipopolysaccharide
MHC – major histocompatibility complex
MSM – men who have sex with men
mRNA – messenger ribonucleic acid
PCR – polymerase chain reaction
PHAC – Public Health Agency of Canada
PE - phycoerythrin
PI – principal investigator
NIH – National Institute of Health
NK – natural killer
TA – annealing temperature
TBE – Tris-Borate-EDTA
TM melting temperature
TNF-α – Tumor Necrosis Factor alpha

RA – research associate
SSO – sequence specific oligonucleotide
SSP – sequence specific primer
STBBI – sexually transmitted blood borne infections
UV – ultraviolet

Appendix B: Letters from Health Research Ethics Board: Solvent Project

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

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APPROVAL FORM

Principal Investigator: Dr. K. Fowke

Ethics Reference Number: H2010:380
Date of REB Meeting: November 22, 2010
Date of Approval: January 13, 2011
Date of Expiry: November 22, 2011

Protocol Title: Sniffing around the Issue: A preliminary investigation into the relationship between solvent use and HIV risk

The following is/are approved for use:

- **Protocol, Version dated 2010/11/08**
- **Research Participant Information and Consent Form, Version 2.0 dated December 20, 2010**
- **Advertisement, Version dated 2010/11/08**
- **Guides – all versions dated 2010/11/08**
  - **Focus Group guide**
  - **Individual interview guide – solvent users**
  - **Individual interview guide – key informants**

The above was approved by Dr. John Arnett, Ph.D., C. Psych., Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated January 10, 2011. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada.*

This approval is valid for one year from the date of the REB meeting at which the study was reviewed. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval must be sought from the relevant institution, if required.

Sincerely yours,

[redacted]

John Arnett, Ph.D., C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255 / Fax: (204) 789-3414

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APPROVAL FORM

Principal Investigator: Dr. K. Fowke

Ethics Reference Number: H2010:380
Date of Approval: November 22, 2011
Date of Expiry: November 22, 2012

Protocol Title: Sniffing around the Issue: A preliminary investigation into the relationship between solvent use and HIV risk

The following is/are approved for use:

- **Annual Approval**
- **Research Participant Information and Consent Form, Version dated 2011/10/10**

The above was approved by Dr. John Arnett, Ph.D., C. Psych., Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your submission dated October 11, 2011. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations of Canada.*

This approval is valid until the expiry date only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval must be sought from the relevant institution, if required.

Sincerely yours,

John Arnett, PhD., C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above Ethics Reference Number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone**: (204) 789-3255 / Fax: (204) 789-3414

www.umanitoba.ca/medicine/ethics

Appendix C: Participant/Key Informant Informed Consent Form: Solvent Project

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Research Participant Information and Consent Form

Title of study: Sniffing around the Issue: A preliminary investigation into the relationship between solvent use and HIV risk

Principle Investigator: Dr. Keith R. Fowke, University of Manitoba, Department of Medical Microbiology, 539-745 Bannatyne Ave. Winnipeg, MB, CANADA tel. (204) 789 – 3818 fax (204) 789-3926

Co-Investigator:
Margaret Ormond, Acting House Manager, Sunshine House, 646 Logan Ave. Winnipeg, MB, CANADA.
Dr. John Wylie, Cadham Provincial Laboratory, Winnipeg, MB
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Introduction:
You are being asked to participate in a focus group or an individual interview as part of a research study. Please take the time to become familiar with this consent form and discuss any questions you may have with the study staff. You make take your time to make your decision about participating in this research study and you may discuss it with your regular doctor, friends, and family before you make your decision. This consent form may have words that you do not understand. Please ask the study staff to stop and explain any words or information that you do not clearly understand. If you decide to join this study, you are free to withdraw from it at anytime.

Purpose of Study:
This research study is being conducted to study solvent use in Manitoba. We are aware that solvent use is a growing problem in Manitoba, especially in street-involved individuals. We are concerned about the health impacts of this practice, including the impact on the immune system's ability to respond to infections such as HIV, influenza, and tuberculosis. We would like to know your views and opinions regarding solvent use as well as health concerns related to solvent use. We also want to know habits regarding solvent abuse. Lastly, we want to know if there is interest among solvent users to be involved in later studies. A total of 29 individuals will participate in this study.

Study Procedure:
We are asking you to help us learn more about solvent use in Manitoba. We are inviting you to take part in this research project. If you accept, you will be asked to take part in a discussion with 6-7 other participants with similar experiences or an individual interview. Individual interviews with solvent users will require two meetings with each individual. Individual interviews with key informants will require one meeting.

Focus Group Procedure:
The group discussion will start with the moderator making sure that you are comfortable. We can also answer questions that you might have about the research. Then we will ask you questions regarding your concerns and perspectives regarding solvent use. You do not have to share any information that you are not comfortable with sharing.

The discussion will take place at Sunshine House. During the discussion the only other people present will be the other participants and the research team. If everyone is comfortable with the discussion being tape-recorded, the session will be tape-recorded. If not, a written account of the discussion will be transcribed. The information recorded by either method is confidential and no else but the research team will have access to the information

Interview Procedure:
The interview will begin with the interviewer making sure that you are comfortable. We can also answer questions about the research that you might have. Then we will ask you questions about your personal history regarding solvent use. You do not have to share any information that you are not comfortable with sharing.

The interview will take place at Sunshine House. During the interview the only other person present will be the interviewer. If you are comfortable with the discussion being tape-recorded, the session will be tape-recorded. If not, a written account of the discussion will be transcribed. The information recorded by either method is confidential and no one else but the research team will have access to the information.

Risks and Discomforts:
There is a risk that you may share some personal or confidential information by chance, or that you may feel uncomfortable or anxious due to the sensitive nature of the topic. We hope that this does not happen. You do not have to answer any questions or take part in a discussion if you feel the questions are too personal or if you feel uncomfortable.

Benefits:
There will be no direct benefit to you from participating in this study. We hope the information learned from this study will benefit your community in the future.

Costs:
There will be no costs to you.

Payment for Participation:
You will be provided with $25 cash in compensation for your out-of-pocket expenses and your time. Each participant will also receive two bus tickets to cover travel expenses. During the focus group, participants will be provided with a hot meal. During the individual interviews, participants will be provided with refreshments.

Confidentiality:
Each study participant will be assigned a unique study number. Information gathered in this research study may be published or presented in public forums, however, your name and other identifying information will not be used or revealed. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include the University of Manitoba Research Ethics Board.

For focus group participants, we will ask you and others in the group not to talk to people outside of the group regarding this discussion and we ask that you keep the information confidential. We, however, cannot stop or prevent participants who were in the group from sharing things that should be confidential

Study information will be kept for 15 years. After this period of time hard copies will be shredded, computer files will be deleted, and recordings will be destroyed.

Voluntary Participating/Withdrawal from the Study
Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision not to participate or to withdraw from this study will not affect your care at Sunshine House. If the study staff feels it is in your best interest to withdraw you from the study, they will remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

Medical Care for Injury Related to the Study
In the case of illness resulting from this study, necessary medical treatment will be available at no cost to you.

Questions:
If you have any questions during or after the study about your treatment or your rights as a research participant you are encouraged to direct them to the study principle investigator Dr. Keith Fowke (204) 789-3818, 539-745 Bannatyne Ave.

For questions about your rights as a research participant, you may contact The University of Manitoba, Bannatyne Campus Research Ethics Board Office at (204) 789-3389

Do not agree to this study unless you have had a chance to ask questions and have received satisfactory answers to all your questions.

Statement of Consent:

I have been asked to take part in: (please check one)

- ☐ Focus Group
- ☐ Individual Interview (Solvent User)
- ☐ Individual Interview (Key Informant)

I have read this consent form. I have had the opportunity to discuss this research study with Dr. Keith Fowke and or his study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statements or implied statements. Any relationship (such as employer, supervisor or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of any of my records that relate to this study by The University of Manitoba Research Ethics Board for quality assurance purposes.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature_____ **Date** _____
(day/month/year)

Participant printed name: _____

I, the undersigned, attest that the information in the Participant Information and Consent Form was accurately explained to and apparently understood by the participant or the participant's legally acceptable representative and that consent to participate in this study was freely given by the participant or the participant's legally acceptable representative.

Witness signature_____ **Date** _____
(day/month/year)

Witness printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____ **Date** _____
(day/month/year)

Signature: _____

Role in the study: _____

Appendix D: Focus Group Interview Tool

Focus Group Guide
Theme: Concerns and Perspectives

1. What are your main concerns?
    a. How does health factor in?
    b. What are your concerns about diseases? (HIV, TB, HCV)
2. Tell us about the reaction of other people to your sniffing
    a. Family, friends, other people on the street, agencies, the public, reserve
3. This is the beginning of a longer research study – What are your suggestions about how we should go about this?
    a. Should it be here (Sunshine House)?
    b. How do you feel about research being done?
    c. Participating in research would require you to come to meetings sober, would this be hard for you?
    d. Is it hard for you to talk about these things? (with other people, when high/sober?)
    e. At some point we will want to look at your blood – How do you feel about that?
4. Other people that we have talked to have spoken about treatment for solvent use. Can you talk about that?
5. Wrap up:
    a. Is there anything that you would like to talk about that we did not cover?
    b. How do you feel about what we talked about today?

Appendix E: Individual Interview Tool: Solvent User Group

Individual Interviews – Solvent Users - Guide

Interview 1
Theme: Personal History

Can you talk about when you started?

1. Who introduced you?
2. Where were you?
3. Who were you with?
4. How old were you?
5. At first, why did you start? (Curiosity? Peer pressure?)
6. Was sniffing connected to anything else?

Community Context

7. Was it something that you had known about before – was it common/ordinary phenomena where you were? (was it normal?)
8. Was anybody against it?
9. Did you have to go someplace different? Did you have to hide?
10. Did you sniff with the same people you hung out with?
11. What was the reaction of other people to you sniffing?

Patterns/Habits/Concerns

12. At what point was it a habit? At what point was it something that you did regularly?
13. Did you ever worry about negative health risks? (Did you worry that it could/would make you sick?)
14. Did you worry about disease (HIV, TB, HCV)?

Wrap –up:

15. How do you feel about what we talked about today?
16. will you come back?
17. Is there something that we can do better? (better food? Shorter? Less people? One interviewer? No taping? An elder present?)
18. Are there things that you want to talk about that we haven't asked?

Interview 2
Theme: Current Experience and Patterns of Use – Social Networks

Can you talk about using?

1. How does it start?
2. Are there periods of time that you are more/less likely to sniff?
3. How often do you sniff? What starts that off?
4. Are there places that you go to sniff?
5. What do you sniff?
6. Would you rather sniff than do anything else? (Is sniff the primary drug of choice or the fallback?)
7. What stops it? (out of money? Out of solvent?)

Social Network

8. Is it something that you do alone? Is it a group thing?
9. Who do you sniff with?
10. Do you sniff with different people than you do other things with?
11. Is it a special event or an ordinary thing?

Personal Experience

12. What does it feel like?
    a. What is the best part of it?
    b. Does it make you want to get more high – and if it does, does that mean you sniff more or use another drug? (what is the pattern?)
    c. Does it make you horny? Is sex different?
13. Are there things that you can't do because you're sniffing? (Can you go home?)
14. Is sniffing connected to anything else? (does getting drunk make you want to get high?)
15. If you wanted to stop sniffing, what do you do?
16. What is the reaction of other people to your sniffing?

Concerns

17. What are your current concerns?
18. Where does health fit into that?
19. Are you concerned with disease (HIV, TB, HCV)?
20. What are your experiences (direct and indirect) with disease?

Research

21. This is the beginning of a longer research study – What are your suggestions about how we should go about this?
22. Should it be here (Sunshine House)?
23. How do you feel about research being done?
24. Participating in research would require you to come to meetings sober, would this be hard for you?
25. Is it hard for you to talk about these things? (with other people, when high/sober?)
26. At some point we will want to look at your blood – How do you feel about that?

Treatment

27. Other people that we have talked to have spoken about treatment for solvent use. Can you talk about that?

Wrap –up:

28. How do you feel about what we talked about today?
29. Is there something that we can do better? (better food? Shorter? Less people? One interviewer? No taping? An elder present?)
30. Are there things that you want to talk about that we haven't asked?

Appendix F: Individual Interview Tool: Key Informant Group

Individual Interview - Key Informants – Guide

1. What is your personal and/or professional connection with solvent users?
    a. How has that connection evolved over time
2. What are, or have been, the barriers to you or your organization when working with solvent users?
    a. Politically, practically, bureaucratically?
3. What is your experience with disease in this population?
    a. Can you talk about your experience with HVI or other diseases?
    b. Where does HIV rank amongst other concerns and other health concerns?
4. Can you talk about treatment options for solvent users in Winnipeg?
5. Is there anything else you would like to talk about today that I have not asked about?

Appendix G: SSP Results

SSP HLA typing results from forty-seven HLA-B35 HIV+ patients enrolled from Manitoba (2008-2010)			
Sample ID	**Low Res**	**Allele 1**	**Allele 2**
P10-0043	B35 B51	Not readable/bad gel	
P10-0137	B35 B35	B*35:XX9 XX9:=:35:01/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94	B*35:XX10 XX10:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159
P10-0172	B08 B35	No allele matches	
P10-0956	B35 B51	B*35:XX2 XX2:=:35:01/35:07/35:130N/35:134N/35:20/35:29/35:32/35:37/35:40N/35:41/35:42/35:48/35:50/35:52/35:53N/35:54/35:57/35:64/35:68/35:76/35:77/35:78/35:82/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*51:XX3 XX3:=:51:01/51:02/51:03/51:06/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:29/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:49/51:50/51:51/51:52/51:53/51:57/51:58/51:59/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:86/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105/51:109
P10-1188	B35 B44	B*35:XX10 XX10:=:35:01/35:130N/35:134N/35:42/35:48/35:50/35:52/35:54/35:57/35:68/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*44:XX11 XX11:=:44:03/44:07/44:108N/44:13/44:26/44:29/44:30/44:32/44:35/44:36/44:37/44:38/44:39/44:40/44:41/44:46/44:50/44:57/44:60/44:61N/44:65/44:69/44:81/44:85/44:92/44:94/44:96/44:98/44:99/44:103/44:105/44:109/44:111/44:114/44:115/44:120/44:122/44:125
P10-1226	B35 B35	B*35:XX12 XX12:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	B*35:XX13 XX13:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159
P10-1236	B15 B35	B*35:XX4 XX4:=:35:01/35:11/35:134N/35:130N/35:32/35:42/35:43/35:48/35:50/35:52/35:53N/35:54/35:57/35:64/35:67/35:68/35:79/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*15:XX3 XX3:=:15:01N/15:01/15:03/15:04/15:08/15:11/15:12/15:149N/15:15/15:16/15:17/15:182N/15:181N/15:18/15:19/15:190N/15:209N/15:20/15:21/15:23/15:24/15:25/15:26N/15:28/15:32/15:34/15:35/15:36/15:38/15:39/15:40/15:43/15:44/15:47/15:49/15:50/15:53/15:54/15:56/15:57/15:60/15:62/15:64/15:65/15:66/15:67/15:71/15:74/15:75/15:79N/15:80/15:81/15:82/15:87/15:92/15:93/15:94N/15:95/15:96/15:97/15:98/15:102/15:103/15:104/15:105/15:110/15:113/15:115/15:117/15:118/15:119/15:120/15:122/15:125/15:127/15:128/15:129/15:132/15:133/15:134/15:135/15:137/15:138/15:140/15:142/15:145/15:146/15:147/15:148/15:152/15:153/15:156/15:157/15:158/15:159/15:160/15:163/15:164/15:165/15:166/15:167/15:171/15:172/15:173/15:174/15:175/15:176/15:177/15:178/15:183/15:184/15:187/15:191/15:192/15:193/15:196/15:197/15:198/15:201/15:203/15:204/15:205/15:206/15:208/15:210/15:211/15:212/15:215/15:216/15:217/15:219/15:221
P10-1675	B35 B51	B*35:XX1 XX1:=:35:01/35:50/35:52/35:54/35:57	B*51:XX2 XX2:=:51:01/51:02/51:03/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:30/51:32/51:33/51:35/51:38/51:39/51:4

			1N/51:43/51:48/51:50/51:51/51:52/51:53/51:57/51:58/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105
P10-2075	B35 B35	B*35:XX9 XX9:=:35:01/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94	B*35:XX10 XX10:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159
P09-0351	B08 B35	B*35:XX2 XX2:=:35:01/35:29/35:50/35:52/35:54/35:57	B*08:XX1 XX1:=:08:07/08:08N
P09-0930	B27 B35	No allele matches	
P09-0186	B27 B35	B*35:XX8 XX8:=:35:01/35:50/35:52/35:53N/35:54/35:57/35:71	B*27:XX7 XX7:=:27:14/27:23/27:41
P09-1405	B27 B35	B*35:XX8 XX8:=:35:01/35:20/35:23/35:50/35:52/35:53N/35:54/35:57	B*27:XX7 XX7:=:27:14/27:23/27:41
P08-1076	B35 B51	No allele matches	
P08-0254	B35 B40	B*35:XX2 XX2:=:35:01/35:41/35:42/35:50/35:52/35:53N/35:54/35:57	B*40:XX3 XX3:=:40:01/40:06/40:07/40:11/40:118N/40:12/40:14/40:155N/40:21/40:22N/40:46/40:47/40:49/40:53/40:54/40:55/40:61/40:62/40:65/40:66/40:67/40:69/40:70/40:72/40:73/40:74/40:75/40:76/40:79/40:81/40:83/40:84/40:88/40:93/40:96/40:99/40:100/40:101/40:102/40:103/40:108/40:109/40:110/40:112/40:113/40:114/40:116/40:117/40:123/40:125/40:127/40:130/40:134/40:135/40:136/40:138/40:139/40:140/40:141/40:146/40:147/40:149/40:150/40:151/40:152/40:153/40:154/40:156/40:162
P08-1507	B35 B51	B*35:XX1 XX1:=:35:01/35:50/35:52/35:54/35:57	B*51:XX2 XX2:=:51:01/51:02/51:03/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:50/51:51/51:52/51:53/51:57/51:58/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105
P08-0527	B35 **	B*35:XX11 XX11:=:35:01/35:11/35:134N/35:130N/35:25/35:42/35:43/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:67/35:68/35:77/35:78/35:79/35:82/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:124/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*35:XX12 XX12:=:35:01/35:11/35:134N/35:130N/35:145N/35:25/35:42/35:43/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:67/35:68/35:77/35:78/35:79/35:82/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:118/35:119/35:120/35:121/35:122/35:123/35:124/35:126/35:131/35:132/35:133/35:135/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159
P08-0755	B35 B44	B*35:XX1 XX1:=:35:01/35:130N/35:134N/35:42/35:48/35:50/35:52/35:54/35:57/35:68/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*44:XX2 XX2:=:44:02S/44:02/44:06/44:08/44:09/44:11/44:19N/44:22/44:23N/44:24/44:27/44:33/44:34/44:44/44:48/44:49/44:52N/44:53/44:55/44:59/44:63/44:66/44:67/44:68/44:71/44:72/44:73/44:74/44:80/44:84/44:86/44:87/44:88/44:89/44:91/44:93/44:95/44:101/44:102/44:104/44:112/44:113/44:116/44:117/44:118/44:119/44:121/44:126/44:127
P08-1150	B27 B35	B*35:XX8 XX8:=:35:01/35:50/35:52/35:53N/35:54/35:57	B*27:XX7 XX7:=:27:14/27:23/27:41
P08-0379	B35 B40	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*40:XX3 XX3:=:40:01/40:06/40:07/40:11/40:118N/40:12/40:14/40:155N/40:21/40:22N/40:46/40:47/40:49/40:53/40:54/40:55/40:61/40:62/40:65/40:66/40:67/40:69/40:70/40:72/40:73/40:74/40:75/40:76/

			40:79/40:81/40:83/40:84/40:88/40:93/40:96/40:99/40:100/40:101/40:102/40:103/40:108/40:109/40:110/40:112/40:113/40:114/40:116/40:117/40:123/40:125/40:127/40:130/40:134/40:135/40:136/40:138/40:139/40:140/40:141/40:146/40:147/40:149/40:150/40:151/40:152/40:153/40:154/40:156/40:162
P08-0232	B35 B51	B*35:XX1 XX1:=:35:01/35:50/35:52/35:54/35:57	B*51:XX2 XX2:=:51:01/51:02/51:03/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:50/51:51/51:52/51:53/51:57/51:58/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105
P08-1186	B35 B51	B*35:XX1 XX1:=:35:01/35:50/35:52/35:54/35:57	B*51:XX2 XX2:=:51:01/51:13/51:63/51:106
P08-0162	B35 B15	B*35:XX4 XX4:=:35:01/35:11/35:130N/35:134N/35:42/35:43/35:50/35:52/35:53N/35:54/35:57/35:64/35:67/35:68/35:79/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*15:XX3 XX3:=:15:01N/15:01/15:03/15:04/15:08/15:09/15:10/15:11/15:12/15:149N/15:15/15:16/15:17/15:182N/15:181N/15:18/15:19/15:190N/15:209N/15:20/15:21/15:23/15:24/15:25/15:26N/15:28/15:32/15:34/15:35/15:36/15:37/15:38/15:39/15:40/15:43/15:44/15:47/15:49/15:50/15:53/15:54/15:56/15:57/15:60/15:62/15:63/15:64/15:65/15:66/15:67/15:71/15:74/15:75/15:79N/15:80/15:81/15:82/15:87/15:90/15:92/15:93/15:94N/15:95/15:96/15:97/15:98/15:99/15:102/15:103/15:104/15:105/15:110/15:113/15:115/15:117/15:118/15:119/15:120/15:122/15:125/15:127/15:128/15:129/15:132/15:133/15:134/15:135/15:137/15:138/15:140/15:142/15:145/15:146/15:147/15:148/15:152/15:153/15:156/15:157/15:158/15:159/15:160/15:163/15:164/15:165/15:166/15:167/15:171/15:172/15:173/15:174/15:175/15:176/15:177/15:178/15:183/15:184/15:187/15:191/15:192/15:193/15:196/15:197/15:198/15:201/15:203/15:204/15:205/15:206/15:208/15:210/15:211/15:212/15:215/15:216/15:217/15:219/15:221
P08-1120	B35 B53	B*35:XX15 XX15:=:35:01/35:07/35:20/35:50/35:52/35:53N/35:54/35:57/35:64	B*53:XX16 XX16:=:53:01/53:03/53:05/53:09/53:10/53:12/53:13/53:15/53:18/53:20/53:21
P08-2083	B35 B38	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*38:XX3 XX3:=:38:01/38:02/38:03/38:04/38:08/38:09/38:11/38:13/38:14/38:15/38:16/38:17/38:18/38:20/38:21/38:22/38:23/38:24/38:25/38:26/38:27
P08-0663	B07 B35	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*07:XX1 XX1:=:07:02/07:03/07:04/07:10/07:111N/07:13/07:15/07:16/07:21/07:22/07:23/07:26/07:27/07:28/07:30/07:33/07:35/07:36/07:37/07:39/07:41/07:42/07:44/07:45/07:46/07:47/07:49N/07:50/07:51/07:52/07:54/07:55/07:56/07:57/07:58/07:59/07:61/07:62/07:63/07:66/07:67N/07:70/07:71/07:73/07:74/07:75/07:76/07:79/07:81/07:82/07:83/07:85/07:87/07:88/07:89/07:91/07:92/07:93/07:94/07:96/07:98/07:99/07:101/07:102/07:103/07:104/07:106/07:107/07:108/07:109/07:113/07:114/07:115/07:116/07:117/07:118/07:119/07:120/07:121/07:122/07:124
P08-0403	B35 B15	B*35:XX4 XX4:=:35:01/35:11/35:134N/35:130N/35:42/35:43/35:50/35:52/35:53N/35:54/35:57/35:64/35:67/35:68/35:79/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:119/35:120/35:122/35:123/35:126/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148/35:159	B*15:XX3 XX3:=:15:01N/15:01/15:03/15:04/15:08/15:11/15:12/15:149N/15:15/15:16/15:17/15:182N/15:181N/15:18/15:19/15:190N/15:209N/15:20/15:21/15:23/15:24/15:25/15:26N/15:28/15:32/15:34/15:35/15:36/15:38/15:39/15:40/15:43/15:44/15:47/15:49/15:50/15:52/15:53/15:54/15:56/15:57/15:60/15:62/15:64/15:65/15:66/15:67/15:71/15:74/15:75/15:79N/15:80/15:81/15:82/15:87/15:92/15:93/15:94N/15:95/15:96/15:97/15:98/15:102/15:103/15:104/15:105/15:110/15:113/15:114/15:115/15:117/15:118/15:119/15:120/15:122/15:124/15:125/15:127/15:128/15:129/15:132/15:133/15:134/15:135/15:137/15:138/15:140/15:142/15:145/15:146/15:147/15:148/15:152/15:153/15:156/15:157/15:158/15:159/15:160/15:163/15:164/15:165/15:166/15:167/15:171/15:172/15:173/15:174/15:175/15:176/15:177/15:178/15:183/15:184/15:187/15:19

			1/15:192/15:193/15:196/15:197/15:198/15:201/15:203/15:204/15:205/15:206/15:208/15:210/15:211/15:212/15:215/15:216/15:217/15:219/15:221
P08-0172	B35 B39	B*35:XX4 XX4:=:35:01/35:03/35:49/35:50/35:52/35:54/35:55/35:57/35:70	B*39:XX5 XX5:=:39:01L/39:01/39:02/39:05/39:07/39:09/39:10/39:13/39:15/39:16/39:19/39:20/39:22/39:23/39:25N/39:26/39:27/39:28/39:31/39:38Q/39:39/39:40N/39:41/39:42/39:44/39:45/39:46/39:47/39:48/39:49/39:51/39:52/39:53/39:54/39:55/39:56/39:59/39:61
P08-0644	B35 B41	B*35:XX5 XX5:=:35:01/35:49/35:50/35:52/35:54/35:57	B*41:XX6 XX6:=:41:02/41:11/41:15
P08-0111	B07 B35	B*35:08	B*07:XX1 XX1:=:07:02/07:03/07:04/07:10/07:111N/07:13/07:15/07:16/07:21/07:22/07:23/07:26/07:27/07:28/07:30/07:33/07:35/07:36/07:37/07:39/07:41/07:42/07:44/07:45/07:46/07:47/07:49N/07:50/07:51/07:52/07:54/07:55/07:56/07:57/07:58/07:59/07:61/07:62/07:63/07:66/07:67N/07:70/07:71/07:73/07:74/07:75/07:76/07:79/07:81/07:82/07:83/07:85/07:87/07:88/07:89/07:91/07:92/07:93/07:94/07:96/07:98/07:99/07:101/07:102/07:103/07:104/07:106/07:107/07:108/07:109/07:113/07:114/07:115/07:116/07:117/07:118/07:119/07:120/07:121/07:122/07:124
P08-0018	B18 B35	B*35:XX5 XX5:=:35:03/35:55/35:70	B*18:XX4 XX4:=:18:01/18:02/18:03/18:04/18:05/18:06/18:08/18:09/18:13/18:17N/18:18/18:20/18:23N/18:24/18:25/18:27/18:28/18:29/18:31/18:32/18:33/18:34/18:37/18:38/18:39/18:40/18:41/18:42/18:43/18:44/18:45/18:46/18:47/18:50/18:51/18:52/18:53/18:54/18:55/18:59
P08-0737	B35 B41	Taq issue	
P08-0756	B35 B44	B*35:XX10 XX10:=:35:01/35:130N/35:134N/35:42/35:48/35:50/35:52/35:54/35:57/35:68/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*44:XX11 XX11:=:44:03/44:07/44:108N/44:13/44:26/44:29/44:30/44:32/44:35/44:36/44:37/44:38/44:39/44:40/44:41/44:46/44:50/44:57/44:60/44:61N/44:65/44:69/44:81/44:85/44:92/44:94/44:96/44:98/44:99/44:103/44:105/44:109/44:111/44:114/44:115/44:120/44:122/44:125
P08-0645	B35 B5701	B*35:XX9 XX9:=:35:53N/35:64	B*57:XX10 XX10:=:57:01/57:06/57:08/57:10/57:11/57:15/57:16/57:18/57:20/57:21/57:23/57:27/57:29/57:31/57:32/57:33/57:34/57:35/57:36/57:37/57:38/57:40/57:41
P08-1048	B35 B44	B*35:XX1 XX1:=:35:01/35:130N/35:134N/35:41/35:42/35:48/35:50/35:52/35:54/35:57/35:68/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*44:XX2 XX2:=:44:02S/44:02/44:06/44:08/44:09/44:11/44:19N/44:22/44:23N/44:24/44:27/44:33/44:34/44:44/44:48/44:49/44:52N/44:53/44:55/44:59/44:63/44:66/44:67/44:68/44:71/44:72/44:73/44:74/44:80/44:84/44:86/44:87/44:88/44:89/44:91/44:93/44:95/44:101/44:102/44:104/44:112/44:113/44:116/44:117/44:118/44:119/44:121/44:126/44:127
P08-0693	B35	B*35:XX9 XX9:=:35:01/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94	B*35:XX10 XX10:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159
P08-1877	B27 B35	B*35:XX8 XX8:=:35:01/35:50/35:52/35:53N/35:54/35:57	B*27:XX7 XX7:=:27:14/27:23/27:41
P08-1308	B35 B62	B*35:XX4 XX4:=:35:01/35:11/35:130N/35:134N/35:42/35:43/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:67/35:68/35:71/35:79/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:119/35:120/35:122/35:123/35:132/35:137/35:138	B*15:XX3 XX3:=:15:01N/15:01/15:03/15:04/15:08/15:11/15:12/15:149N/15:14/15:15/15:16/15:17/15:18/15:182N/15:181N/15:190N/15:19/15:209N/15:20/15:21/15:23/15:24/15:25/15:26N/15:28/15:32/15:34/15:35/15:36/15:38/15:39/15:40/15:43/15:44/15:47/15:49/15:50/15:53/15:54/15:56/15:57/15:60/15:62/15:64/15:65/15:66/15

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P08-1943	B27 B35	B*35:XX7 XX7:=:35:03/35:55/35:70	B*27:XX6 XX6:=:27:14/27:23
P08-0584	B35 B58	Plate not readable	
P08-1760	B07 B35	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*07:XX1 XX1:=:07:02/07:03/07:04/07:10/07:111N/07:13/07:15/07:16/07:21/07:22/07:23/07:26/07:27/07:28/07:30/07:33/07:35/07:36/07:37/07:39/07:41/07:42/07:44/07:45/07:46/07:47/07:49N/07:50/07:51/07:52/07:54/07:55/07:56/07:57/07:58/07:59/07:61/07:62/07:63/07:66/07:67N/07:70/07:71/07:73/07:74/07:75/07:76/07:79/07:81/07:82/07:83/07:85/07:87/07:88/07:89/07:91/07:92/07:93/07:94/07:96/07:98/07:99/07:101/07:102/07:103/07:104/07:106/07:107/07:108/07:109/07:113/07:114/07:115/07:116/07:117/07:118/07:119/07:120/07:121/07:122/07:124
P08-0088	B07 B35	B*35:XX2 XX2:=:35:03/35:55/35:70	B*07:XX1 XX1:=:07:02/07:03/07:04/07:10/07:111N/07:13/07:15/07:16/07:21/07:22/07:23/07:26/07:27/07:28/07:30/07:33/07:35/07:36/07:37/07:39/07:41/07:42/07:44/07:45/07:46/07:47/07:49N/07:50/07:51/07:52/07:54/07:55/07:56/07:57/07:58/07:59/07:61/07:62/07:63/07:66/07:67N/07:70/07:71/07:73/07:74/07:75/07:76/07:79/07:81/07:82/07:83/07:85/07:87/07:88/07:89/07:91/07:92/07:93/07:94/07:96/07:98/07:99/07:101/07:102/07:103/07:104/07:106/07:107/07:108/07:109/07:113/07:114/07:115/07:116/07:117/07:118/07:119/07:120/07:121/07:122/07:124
P08-0656	B35 B47	B*35:XX7 XX7:=:35:01/35:23/35:50/35:52/35:54/35:57	B*47:XX8 XX8:=:47:01/47:02/47:03/47:06/47:07/47:08
P08-1224	B35 B14	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*14:XX1 XX1:=:14:02/14:03/14:04/14:06/14:09/14:15/14:16/14:17/14:18/14:20
P08-0688	B35 B51	B*35:XX1 XX1:=:35:03/35:55/35:70	B*51:XX2 XX2:=:51:01/51:02/51:03/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:50/51:51/51:52/51:53/51:57/51:58/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105
P08-1331	B3502 B35	B*35:XX2 XX2:=:35:01/35:04/35:130N/35:134N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*35:XX3 XX3:=:35:02/35:129N/35:146
P08-0163	B35 B35	B*35:XX8 XX8:=:35:01/35:130N/35:134N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:122/35:123/35:126/35:131/35:132/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	B*35:XX9 XX9:=:35:03/35:55/35:65Q/35:70/35:74/35:75/35:84/35:85/35:98/35:106/35:127/35:128/35:150/35:151/35:152/35:153/35:155/35:160

Appendix H: SSO Results

SSO HLA typing results from forty-seven HLA-B35 HIV+ patients enrolled from Manitoba (2008-2010).			
Sample ID	**Low Res**	**Allele 1**	**Allele 2**
P10-0043	B35 B51	B*35:REEZ REEZ:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:103/35:108/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:144/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*51:REPK REPK:=:51:01/51:11N/51:24/51:30/51:32/51:48/51:51/51:94/51:95/51:96/51:98N/51:100/51:105/51:107/51:109/51:110N/51:111/51:113/51:117/51:121/51:123/51:124/51:125/51:126
P10-0137	B35 B35	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177
P10-0172	B08 B35	**SSP not clear** B*35:05	 B*08:RBBY RBBY:=:08:01/08:19N/08:58/08:59/08:63/08:72N/08:73
P10-0956	B35 B51	**SSP NOT CLEAR** B*35:REEZ REEZ:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:103/35:108/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:144/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	 B*51:REPK REPK:=:51:01/51:11N/51:24/51:30/51:32/51:48/51:51/51:94/51:95/51:96/51:98N/51:100/51:105/51:107/51:109/51:110N/51:111/51:113/51:117/51:121/51:123/51:124/51:125/51:126
P10-1188	B35 B44	B*35:CCUA CCUA:=:01/57	B*44:MDBK MDBK:=:03/114/115/122/125
P10-1226	B35 B35	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177
P10-1236	B15 B35	B*35:XX2 XX2:=:35:01/35:43/35:57/35:67/35:79/35:102	B*15:XX1 XX1:=:15:01/15:01N/15:20/15:102/15:104/15:140/15:146/15:183/15:187/15:190N/15:201/15:205/15:206/15:211/15:227/15:228/15:232/15:234
P10-1675	B35 B51	B*35:CCUA CCUA:=:01/57	B*51:XX1 XX1:=:51:01/51:11N/51:30/51:32/51:48/51:51/51:94/51:96/51:98N/51:100/51:105/51:110N/51:111/51:113/51:121/51:124/51:125/51:126
P10-2075	B35 B35	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177
P09-0351	B08 B35	B*35:CCUA CCUA:=:01/57	B*08:XX1 XX1:=:08:01/08:19N/08:58/08:59/08:63/08:73

P09-0930	B27 B35	**SSP NOT CLEAR** B*35:REFR REFR:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:108/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:141/35:144/35:145N/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84
P09-0186	B27 B35	B*35:CCUA CCUA:=:01/57	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84
P09-1405	B27 B35	B*35:REFR REFR:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:108/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:141/35:144/35:145N/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84
P08-1076	B35 B51	B*35:CGAH CGAH:=:03/70	B*51:KJSR KJSR:=:01/107
P08-0254	B35 B40	B*35:CCUA CCUA:=:01/57	B*40:RPXN RPXN:=:40:01/40:55/40:102/40:116/40:118N/40:134/40:138/40:141/40:150/40:151/40:152/40:153/40:154/40:155N/40:168/40:175/40:179
P08-1507	B35 B51	B*35:CCUA CCUA:=:01/57	B*51:XX1 XX1:=:51:01/51:11N/51:30/51:32/51:48/51:51/51:94/51:96/51:98N/51:100/51:105/51:110N/51:111/51:113/51:121/51:124/51:125/51:126
P08-0527	B35 **	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177
P08-0755	B35 B44	B*35:CCUA CCUA:=:01/57	B*44:XX1 XX1:=:44:02/44:02S/44:27/44:66/44:104/44:118/44:119/44:121/44:126/44:137
P08-1150	B27 B35	B*35:CCUA CCUA:=:01/57	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84
P08-0379	B35 B40	B*35:CCUA CCUA:=:01/57	B*40:RPXN RPXN:=:40:01/40:55/40:102/40:116/40:118N/40:134/40:138/40:141/40:150/40:151/40:152/40:153/40:154/40:155N/40:168/40:175/40:179
P08-0232	B35 B51	B*35:CCUA CCUA:=:01/57	B*51:XX1 XX1:=:51:01/51:11N/51:30/51:32/51:48/51:51/51:94/51:96/51:98N/51:100/51:105/51:110N/51:111/51:113/51:121/51:124/51:125/51:126
P08-1186	B35 B51	B*35:CCUA CCUA:=:01/57	B*51:XX1 XX1:=:51:01/51:11N/51:30/51:32/51:48/51:51/51:94/51:96/51:98N/51:100/51:105/51:110N/51:111/51:113/51:121/51:124/51:125/51:126
P08-0162	B35 B15	B*35:XX2 XX2:=:35:01/35:43/35:57/35:67/35:79/35:102	B*15:XX1 XX1:=:15:01/15:01N/15:20/15:102/15:104/15:140/15:146/15:183/15:187/15:190N/15:201/15:205/15:206/15:211/15:227/15:228/15:232/15:234
P08-1120	B35 B53	B*35:CCUA CCUA:=:01/57	B*53:KJW KJW:=:01/25/26
P08-2083	B35	B*35:CCUA	B*38:REHH

	B38	CCUA:=:01/57	REHH:=:38:01/38:24/38:31/38:34N
P08-0663	B07 B35	B*35:CCUA CCUA:=:01/57	B*07:RDFP RDFP:=:07:02/07:44/07:49N/07:58/07:59/07:61/07:67N/07:101/07:103/07:104/07:106/07:108/07:111N/07:119/07:120/07:121/07:126/07:128/07:129/07:130/07:132/07:136/07:141/07:142/07:144
P08-0403	B35 B15	B*35:XX2 XX2:=:35:01/35:43/35:57/35:67/35:79/35:102	B*15:XX1 XX1:=:15:01/15:01N/15:20/15:102/15:104/15:140/15:146/15:183/15:187/15:190N/15:201/15:205/15:206/15:211/15:227/15:228/15:232/15:234
P08-0172	B35 B39	B*35:CCUA CCUA:=:01/57	B*39:REHU REHU:=:39:01/39:01L/39:05/39:46/39:59/39:67
P08-0644	B35 B41	**ABMIGUOUS** **SSP not clear** B*35:RSXN B*35:63 RSXN:=:35:01/35:29/35:40N/35:42/35:57/35:94/35:101/35:108/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:144/35:145N/35:147/35:161/35:165N/35:168/35:170/35:173N/35:177	 B*41:02 B*35:87
P08-0111	B07 B35	B*35:08	B*07:RDFP RDFP:=:07:02/07:44/07:49N/07:58/07:59/07:61/07:67N/07:101/07:103/07:104/07:106/07:108/07:111N/07:119/07:120/07:121/07:126/07:128/07:129/07:130/07:132/07:136/07:141/07:142/07:144
P08-0018	B18 B35	B*35:CGAH CGAH:=:03/70	B*18:RBDJ RBDJ:=:01/53/55/59/62/63
P08-0737	B35 B41	**SSP NOT CLEAR** B*35:REFK REFK:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:108/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	 B*44:MDBH MDBH:=:02/03/114/115/122/125
P08-0756	B35 B44	B*35:CCUA CCUA:=:01/57	B*44:MDBK MDBK:=:03/114/115/122/125
P08-0645	B35 B5701	B*35:CCUA CCUA:=:01/57	B*57:RBJP RBJP:=:01/29/33/35/36/37/41/44/47/48/52
P08-1048	B35 B44	B*35:CCUA CCUA:=:01/57	B*44:XX1 XX1:=:44:02/44:02S/44:27/44:66/44:104/44:118/44:119/44:121/44:126/44:137
P08-0693	B35	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177
P08-1877	B27 B35	B*35:CCUA CCUA:=:01/57	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84
P08-1308	B35 B62	**SSP NOT CLEAR**	

		B*35:RJVZ RJVZ:=:35:01/35:14/35:29/35:32/35:40N/35:42/35:43/35:57/35:67/35:68/35:79/35:94/35:101/35:102/35:107/35:108/35:116/35:119/35:120/35:121/35:122/35:123/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:144/35:147/35:148/35:161/35:165N/35:168/35:170/35:173N/35:177	B*15:RJTJ RJTJ:=:15:01/15:01N/15:05/15:20/15:70/15:78/15:85/15:102/15:104/15:140/15:146/15:154/15:165/15:183/15:187/15:190N/15:201/15:205/15:206/15:211/15:227/15:228/15:232/15:234
P08-1943	B27 B35	**SSP NOT CLEAR** B*35:REGN REGN:=:03/70/106/127/152/160/181	 B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84
P08-0584	B35 B58	**SSP NOT CLEAR** B*35:REEX REEX:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	 B*58:02
P08-1760	B07 B35	B*35:CCUA CCUA:=:01/57	B*07:RDFP RDFP:=:07:02/07:44/07:49N/07:58/07:59/07:61/07:67N/07:101/07:103/07:104/07:106/07:108/07:111N/07:119/07:120/07:121/07:126/07:128/07:129/07:130/07:132/07:136/07:141/07:142/07:144
P08-0088	B07 B35	B*35:CGAH CGAH:=:03/70	B*07:RDFP RDFP:=:07:02/07:44/07:49N/07:58/07:59/07:61/07:67N/07:101/07:103/07:104/07:106/07:108/07:111N/07:119/07:120/07:121/07:126/07:128/07:129/07:130/07:132/07:136/07:141/07:142/07:144
P08-0656	B35 B47	B*35:XX1 XX1:=:35:01/35:23/35:57	B*47:BC BC:=:02/03
P08-1224	B35 B14	B*35:CCUA CCUA:=:01/57	B*14:EGG EGG:=:02/22
P08-0688	B35 B51	B*35:CGAH CGAH:=:03/70	B*51:XX1 XX1:=:109/117/123
P08-1331	B350 2 B35	B*35:RMGE RMGE:=:35:02/35:129N/35:146/35:182/35:183	B*35:XX1 XX1:=:35:01/35:04/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:130N/35:132/35:134N/35:137/35:138/35:139/35:140/35:144/35:147/35:148/35:161/35:165N/35:168/35:170/35:173N/35:177
P08-0163	B35 B35	**SSP NOT CLEAR** B*35:RJWG RJWG:=:35:01/35:29/35:40N/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:126/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	 B*35:RJXB RJXB:=:03/70/106/127/128/136/152/153/155/160/179/181

Appendix I: Sequencing Results

Full set of results from 167 DNA samples from HIV+ individuals in Manitoba (2007-2010)) HLA typed using direct sequencing. Samples were previously identified as HLA-B35 by SSO typing. Eight highlighted samples were eliminated from final analysis						
Sample ID	**Allele 1**	**Allele 2**		**Sample ID**	**Allele 1**	**Allele 2**
P10-0137	35:01:01G	35:01:01G		P08-0663	07:02:01G	35:01:01G
P08-0254	35:01:01G	40:01:01G		P08-1760	07:02:01G	35:01:01G
P08-0403	15:01:01G	35:01:01G		P09-0138	35:20:01	44:02:01G
P08-0088	07:02:01G	35:03:01G		P08-1283	35:01:01G	57:03:01
P10-0958	07:02:01G	35:01:01G		P08-0369	27:05:02G	35:01:01G
P09-0204	08:01:01G	35:01:01G		P08-0815	35:08:01	40:02:01G
P08-0392	Didn't work			P08-0635	35:03:01G	52:01:01G
P08-0452	35:01:01G	40:01:01G		P07-1497	15:T01:01G	35:01:01G
P10-0172	08:01:01G	35:05:01		P07-0740	35:01:01G	44:03:01G
P08-1507	35:01:01G	51:01:01G		P07-0244	07:02:01G	35:01:01G
P08-0172	35:01:01G	39:01:01G		P07-1940	35:08:01	35:43:01
P08-0656	35:01:01G	47:03		P07-0820	35:01:01G	35:01:01G
P09-1440	35:01:01G	44:03:01G		P07-0537	35:01:01G	51:01:01G
P08-1065	14:02:01	35:01:01G		P08-0426	35:01:01G	35:01:01G
P08-0519	08:01:01G	35:01:01G		P07-0382	27:05:02G	35:01:01G
P10-1188	35:01:01G	44:03:01G		P07-0241	Didn't work	
P08-0527	35:01:01G	35:01:01G		P07-0502	35:01:01G	40:01:01G
P08-0644	35:01:01G	41:02:01		P07-1158	27:05:02G	35:03:01G
P08-1224	14:02:01	35:01:01G		P07-1367	15:01:01G	35:01:01G
P09-1351	Didn't work			P07-0750	Didn't work	
P08-0879	35:01:01G	41:02:01		P07-0288	35:01:01G	40:02:01G
P08-1682	35:01:01G	39:01:01G		P07-0377	35:01:01G	38:02:01G
P10-1226	35:01:01G	35:01:01G		P07-1834	27:05:02G	35:01:01G
P08-0755	35:01:01G	44:02:01G		P07-0725	35:01:01G	44:02:01G
P08-0111	07:02:01G	35:08:01		P07-1154	27:05:02G	35:01:01G
P08-0688	35:03:01G	51:01:01G		P07-0517	27:05:02G	35:01:01G
P09-1868	35:12:01	15:01:01G		P08-0019	15:35	35:30
P08-1578	35:08:01	39:01:01G		P07-1043	18:01:01G	44:02:01G
P08-0297	08:01:01G	35:02:01		P07-1797	08:01:01G	35:01:01G
P10-1236	15:01:01G	35:01:01G		P07-1023	35:01:01G	39:01:01G
P08-1150	27:05:02G	35:01:01G		P07-1405	08:01:01G	35:01:01G
P08-0018	18:01:01G	35:03:01G		P07-0787	14:02:01	35:03:01G
P08-1331	35:01:01G	35:02:01		P07-1299	35:01:01G	40:01:01G
P09-0477	35:03:01G	39:06:02G		P08-0514	15:01:01G	35:03:01G
P08-2047	35:01:01G	40:02:01G		P07-0828	35:01:01G	35:01:01G
P08-1523	35:01:01G	44:03:01G		P07-0742	35:01:01G	48:01:01G
P08-0451	07:02:01G	35:01:01G		P07-1351	18:01:01G	35:01:01G
P10-1675	35:01:01G	51:01:01G		P07-1409	35:01:01G	40:02:01G

P08-0379	35:01:01G	40:01:01G		P07-0672	07:02:01G	35:03:01G
P08-1076	35:03:01G	51:01:01G		P07-0498	35:01:01G	40:02:01G
P08-0737	35:01:01G	44:03:01G		P07-1138	18:01:01G	35:01:01G
P08-0163	35:01:01G	35:03:01G		P07-1271	35:01:01G	51:01:01G
P09-1105	35:01:01G	51:01:01G		P07-1512	15:01:01G	35:01:01G
P08-0012	35:01:01G	40:02:01G		P07-1950	14:01:01	35:43:01G
P08-1474	35:01:01G	35:01:01G		P07-1132	15:01:01G	35:01:01G
P08-1457	35:01:01G	45:01G		P07-0980	35:03:01G	39:10:01
P10-2075	35:01:01G	35:01:01G		P07-0658	15:01:01G	35:03:01G
P08-0232	35:01:01G	51:01:01G		P07-0970	35:01:01G	39:01:01G
P08-0756	35:01:01G	44:03:01G		P07-1153	35:01:01G	44:03:01G
P10-0021	35:02:01	51:01:01G		P07-0755	35:01:01G	47:03
P09-0866	15:01:01G	35:01:01G		P07-1198	08:01:01G	35:03:01G
P08-0574	35:01:01G	48:07		P07-1645	07:05:01G	57:02:01
P08-1576	35:01:01G	44:03:01G		P07-1648	18:01:01G	35:01:01G
P08-1266	35:01:01G	44:02:01G		P07-1112	35:01:01G	48:01:01G
P09-0351	08:01:01G	35:01:01G		P07-1123	27:44	53:03
P08-1186	35:01:01G	51:01:01G		P07-1257	35:01:01G	40:02:01G
P08-0645	35:01:01G	57:01:01G		P07-0929	15:13:01	35:05:01
P09-1709	35:01:01G	57:01:01G		P07-0836	35:01:01G	39:01:01G
P08-1016	27:05:02G	35:01:01G		P07-1726	08:01:01G	35:01:01G
P08-0978	35:01:01G	50:01:01		P07-0812	18:01:01G	35:01:01G
P08-0246	07:02:01G	35:01:01G		P07-0337	13:02:01G	35:01:01G
P09-0930	27:05:02G	35:01:01G		P07-0753	35:01:01G	35:01:01G
P08-0162	15:01:01G	35:01:01G		P07-0384	35:01:01G	51:01:01G
P08-1308	15:01:01G	35:01:01G		P07-1943	35:01:01G	39:01:01G
P09-0527	27:05:02G	35:01:01G		P07-0601	15:01:01G	35:01:01G
P08-1008	15:01:01G	35:01:01G		P07-0737	14:02:01	35:01:01G
P08-0229	13:02:01G	35:03:01G		P07-1208	35:34	35:34
P08-1388	07:02:01G	35:01:01G		P07-0538	35:01:01G	48:01:01G
P09-0186	27:05:02G	35:01:01G		P07-1965	35:01:01G	40:02:01G
P08-1120	35:01:01G	53:01:01		P07-0572	08:01:01G	35:01:01G
P08-1943	27:05:02G	35:03:01G		P07-1149	35:01:01G	35:01:01G
P09-1427	35:01:01G	39:01:01G		P07-1156	27:05:02G	35:01:01G
P08-1085	35:01:01G	51:01:01G		P07-0696	35:01:01G	40:02:01G
P08-0288	15:01:01G	35:01:01G		P07-1938	15:01:01G	35:01:01G
P08-0699	35:01:01G	51:01:01G		P07-1480	35:01:01G	44:02:01G
P09-1405	27:05:02G	35:01:01G		P07-1999	15:01:01G	35:01:01G
P08-2083	35:01:01G	38:01:01G		P07-1066	27:44	53:03
P08-0584	35:01:01G	58:02		P07-0715	15:01:01G	35:01:01G
P09-1282	15:01:01G	35:01:01G		P07-1306	07:02:01G	35:01:01G
P08-0700	08:01:01G	35:01:01G		P07-1973	08:01:01G	35:08:01
P08-0181	35:01:01G	51:01:01G		P07-1455	35:01:01G	40:01:01G
P08-0089	07:02:01G	35:01:01G		P07-1284	07:02:01G	35:01:01G

P07-0556	35:01:01G	40:02:01G		P07-1547	35:01:01G	40:02:01G
P07-0691	15:01:01G	35:03:01G				

Appendix J: Table comparing three types of HLA high resolution typing results

High resolution HLA typing results from HLA-B35 HIV+ patients enrolled from Manitoba (2008-2010). Results show SSP and SSO typing results as well as direct sequencing when adequate sample was available							
Sample	**Low**	**SSP**		**SSO**		**Sequencing**	
ID	**Res**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
P10-0043	B35 B51	Not readable/bad gel		B*35:REEZ REEZ:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:103/35:108/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:144/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*51:REPK REPK:=:51:01/51:11N/51:24/51:30/51:32/51:48/51:51/51:94/51:95/51:96/51:98N/51:100/51:105/51:107/51:109/51:110N/51:111/51:113/51:117/51:121/51:123/51:124/51:125/51:126	Not enough sample for sequencing	
P10-0137	B35 B35	B*35:XX9 XX9:=:35:01/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94	B*35:XX10 XX10:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	35:01:01G	35:01:01G
P10-0172	B08 B35	No allele matches		**SSP not clear** B*35:05	 B*08:RBBY RBBY:=:08:01/08:19N/08:58/08:59/08:63/08:72N/08:73	35:05:01	08:01:01G
P10-0956	B35 B51	B*35:XX2 XX2:=:35:01/35:07/35:130N/35:134N	B*51:XX3 XX3:=:51:01/51:02/51:03/51:06/51:07/51:11N/5	**SSP NOT CLEAR**		Not enough sample for sequencing	

		/35:20/35:29/35:32/35:37/35:40N/35:41/35:42/35:48/35:50/35:52/35:53N/35:54/35:57/35:64/35:68/35:76/35:77/35:78/35:82/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	1:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:29/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:49/51:50/51:51/51:52/51:53/51:57/51:58/51:59/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:86/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105/51:109	B*35:REEZ REEZ:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:103/35:108/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:144/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*51:REPK REPK:=:51:01/51:11N/51:24/51:30/51:32/51:48/51:51/51:94/51:95/51:96/51:98N/51:100/51:105/51:107/51:109/51:110N/51:111/51:113/51:117/51:121/51:123/51:124/51:125/51:126		
P10-1188	B35 B44	B*35:XX10 XX10:=:35:01/35:130N/35:134N/35:42/35:48/35:50/35:52/35:54/35:57/35:68/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*44:XX11 XX11:=:44:03/44:07/44:108N/44:13/44:26/44:29/44:30/44:32/44:35/44:36/44:37/44:38/44:39/44:40/44:41/44:46/44:50/44:57/44:60/44:61N/44:65/44:69/44:81/44:85/44:92/44:94/44:96/44:98/44:99/44:103/44:105/44:109/44:111/44:114/44:115/44:120/44:122/44:125	B*35:CCUA CCUA:=:01/57	B*44:MDBK MDBK:=:03/114/115/122/125	35:01:01G	44:03:01G
P10-1226	B35 B35	B*35:XX12 XX12:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	B*35:XX13 XX13:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	35:01:01G	35:01:01G
P10-1236	B15 B35	B*35:XX4 XX4:=:35:01/35:11/35:134N/35:130N/35:32/35:42/35:43/35:48/35:50/35:52/35:53N/35:54/35:57/35:64/35:67/35:68/35:79/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/3	B*15:XX3 XX3:=:15:01N/15:01/15:03/15:04/15:08/15:11/15:12/15:149N/15:15/15:16/15:17/15:182N/15:181N/15:18/15:19/15:190N/15:209N/15:20/15:21/15:23/15:24/15:25/15:26N/15:28/15:32/15:34/15:35/15:36/15:38/15:39/15:40/15:43/15:44/15:47/15:49/15:50/15:53/15:54/15:56/15:57/15:60/15:62/15:64/15:65/15:66/15:67/15:71/15:74/15:75/15:79N/15:80/15:81/15:82/15:87/15:92/15:93/15:94N/15:95/15:96/15:97/15:98/15:1	B*35:XX2 XX2:=:35:01/35:43/35:57/35:67/35:79/35:102	B*15:XX1 XX1:=:15:01/15:01N/15:20/15:102/15:104/15:140/15:146/15:183/15:187/15:190N/15:201/15:205/15:206/15:211/15:227/15:228/15:232/15:234	35:01:01G	15:01:01G

		5:144/35:147/35:148	02/15:103/15:104/15:105/15:110/15:113/15:115/15:117/15:118/15:119/15:120/15:122/15:125/15:127/15:128/15:129/15:132/15:133/15:134/15:135/15:137/15:138/15:140/15:142/15:145/15:146/15:147/15:148/15:152/15:153/15:156/15:157/15:158/15:159/15:160/15:163/15:164/15:165/15:166/15:167/15:171/15:172/15:173/15:174/15:175/15:176/15:177/15:178/15:183/15:184/15:187/15:191/15:192/15:193/15:196/15:197/15:198/15:201/15:203/15:204/15:205/15:206/15:208/15:210/15:211/15:212/15:215/15:216/15:217/15:219/15:221				
P10-1675	B35 B51	B*35:XX1 XX1:=:35:01/35:50/35:52/35:54/35:57	B*51:XX2 XX2:=:51:01/51:02/51:03/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:50/51:51/51:52/51:53/51:57/51:58/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105	B*35:CCUA CCUA:=:01/57	B*51:XX1 XX1:=:51:01/51:11N/51:30/51:32/51:48/51:51/51:94/51:96/51:98N/51:100/51:105/51:110N/51:111/51:113/51:121/51:124/51:125/51:126	35:01:01G	51:01:01G
P10-2075	B35 B35	B*35:XX9 XX9:=:35:01/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94	B*35:XX10 XX10:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	35:01:01G	35:01:01G
P09-0351	B08 B35	B*35:XX2 XX2:=:35:01/35:29/35:50/35:52/35:54/35:57	B*08:XX1 XX1:=:08:07/08:08N	B*35:CCUA CCUA:=:01/57	B*08:XX1 XX1:=:08:01/08:19N/08:58/08:59/08:63/08:73	35:01:01G	08:01:01G
P09-0930	B27 B35	No allele matches		**SSP NOT CLEAR** B*35:REFR	 B*27:REDU	35:01:01G	27:05:02G

				REFR:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:108/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:141/35:144/35:145N/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	REDU:=:27:05/27:13/27:64N/27:72/27:84		
P09-0186	B27 B35	B*35:XX8 XX8:=:35:01/35:50/35:52/35:53N/35:54/35:57/35:71	B*27:XX7 XX7:=:27:14/27:23/27:41	B*35:CCUA CCUA:=:01/57	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84	35:01:01G	27:05:02G
P09-1405	B27 B35	B*35:XX8 XX8:=:35:01/35:20/35:23/35:50/35:52/35:53N/35:54/35:57	B*27:XX7 XX7:=:27:14/27:23/27:41	B*35:REFR REFR:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:108/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:141/35:144/35:145N/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84	35:01:01G	27:05:02G
P08-1076	B35 B51	No allele matches		B*35:CGAH CGAH:=:03/70	B*51:KJSR KJSR:=:01/107	35:03:01G	51:01:01G
P08-0254	B35 B40	B*35:XX2 XX2:=:35:01/35:41/35:42/35:50/35:52/35:53N/35:54/35:57	B*40:XX3 XX3:=:40:01/40:06/40:07/40:11/40:118N/40:12/40:14/40:155N/40:21/40:22N/40:46/40:47/40:49/40:53/40:54/40:55/40:61/40:62/40:65/40:66/40:67/40:69/40:70/40:72/40:73/40:74/40:75/40:76/40:79/40:81/40:83/40:84/40:88/40:93/40:96/40:99/40:100/40:101/40:102/40:103/40:108/40:109/40:110/40:112/40:113/40:114/40:116/40:117/40:123/40:125/40:127/40:130/40:134/40:135/40:136/40:138/40:139/40:140/40:141/40:146/40:147/40:149/40:150/40:151/40:152/40:153/40:154/40:156/40:162	B*35:CCUA CCUA:=:01/57	B*40:RPXN RPXN:=:40:01/40:55/40:102/40:116/40:118N/40:134/40:138/40:141/40:150/40:151/40:152/40:153/40:154/40:155N/40:168/40:175/40:179	35:01:01G	40:01:01G
P08-1507	B35 B51	B*35:XX1 XX1:=:35:01/35:50/35:52/35:54/35:57	B*51:XX2 XX2:=:51:01/51:02/51:03/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:50/51:51/51:52/51:53/51:57/51:58/51:	B*35:CCUA CCUA:=:01/57	B*51:XX1 XX1:=:51:01/51:11N/51:30/51:32/51:48/51:51/51:94/51:96/51:98N/51:100/51:105/51:110N/51:111/51:1	35:01:01G	51:01:01G

			60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105		13/51:121/51:124/51:125/51:126		
P08-0527	B35 **	B*35:XX11 XX11:=:35:01/35:11/35:134N/35:130N/35:25/35:42/35:43/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:67/35:68/35:77/35:78/35:79/35:82/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:124/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*35:XX12 XX12:=:35:01/35:11/35:134N/35:130N/35:145N/35:25/35:42/35:43/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:67/35:68/35:77/35:78/35:79/35:82/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:118/35:119/35:120/35:121/35:122/35:123/35:124/35:126/35:131/35:132/35:133/35:135/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	35:01:01G	35:01:01G
P08-0755	B35 B44	B*35:XX1 XX1:=:35:01/35:130N/35:134N/35:42/35:48/35:50/35:52/35:54/35:57/35:68/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*44:XX2 XX2:=:44:02S/44:02/44:06/44:08/44:09/44:11/44:19N/44:22/44:23N/44:24/44:27/44:33/44:34/44:44/44:48/44:49/44:52N/44:53/44:55/44:59/44:63/44:66/44:67/44:68/44:71/44:72/44:73/44:74/44:80/44:84/44:86/44:87/44:88/44:89/44:91/44:93/44:95/44:101/44:102/44:104/44:112/44:113/44:116/44:117/44:118/44:119/44:121/44:126/44:127	B*35:CCUA CCUA:=:01/57	B*44:XX1 XX1:=:44:02/44:02S/44:27/44:66/44:104/44:118/44:119/44:121/44:126/44:137	35:01:01G	44:02:01G
P08-1150	B27 B35	B*35:XX8 XX8:=:35:01/35:50/35:52/35:53N/35:54/35:57	B*27:XX7 XX7:=:27:14/27:23/27:41	B*35:CCUA CCUA:=:01/57	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84	35:01:01G	27:05:02G
P08-0379	B35 B40	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*40:XX3 XX3:=:40:01/40:06/40:07/40:11/40:118N/40:12/40:14/40:155N/40:21/40:22N/40:46/40:47/40:49/40:53/40:54/40:55/40:61/40:62/40:65/40:66/40:67/40:69/40:70/40:72/40:73/40:74/40:75/40:76/40:79/40:81/40:83/40:84/40:88/40:93/40:96/40:99/40:100/40:101/40:102/40:103/40:108/40:109/40:110/40:112/40:113/40:114/40:116/40:117/40:123/40:125/40:127/40:130/40:134/40:135/40:136/40:138/40:139/40:140/40:141/40:146/40:147/40:149/40:150/40:151/40:152/40:153/40:154/40:156/40:162	B*35:CCUA CCUA:=:01/57	B*40:RPXN RPXN:=:40:01/40:55/40:102/40:116/40:118N/40:134/40:138/40:141/40:150/40:151/40:152/40:153/40:154/40:155N/40:168/40:175/40:179	35:01:01G	40:01:01G

P08-0232	B35 B51	B*35:XX1 XX1:=:35:01/35:50/35:52/35:54/35:57	B*51:XX2 XX2:=:51:01/51:02/51:03/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:50/51:51/51:52/51:53/51:57/51:58/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105	B*35:CCUA CCUA:=:01/57	B*51:XX1 XX1:=:51:01/51:11N/51:30/51:32/51:48/51:51/51:94/51:96/51:98N/51:100/51:105/51:110N/51:111/51:113/51:121/51:124/51:125/51:126	35:01:01G	51:01:01G
P08-1186	B35 B51	B*35:XX1 XX1:=:35:01/35:50/35:52/35:54/35:57	B*51:XX2 XX2:=:51:01/51:13/51:63/51:106	B*35:CCUA CCUA:=:01/57	B*51:XX1 XX1:=:51:01/51:11N/51:30/51:32/51:48/51:51/51:94/51:96/51:98N/51:100/51:105/51:110N/51:111/51:113/51:121/51:124/51:125/51:126	35:01:01G	51:01:01G
P08-0162	B35 B15	B*35:XX4 XX4:=:35:01/35:11/35:130N/35:134N/35:42/35:43/35:50/35:52/35:53N/35:54/35:57/35:64/35:67/35:68/35:79/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*15:XX3 XX3:=:15:01N/15:01/15:03/15:04/15:08/15:09/15:10/15:11/15:12/15:149N/15:15/15:16/15:17/15:182N/15:181N/15:18/15:19/15:190N/15:209N/15:20/15:21/15:23/15:24/15:25/15:26N/15:28/15:32/15:34/15:35/15:36/15:37/15:38/15:39/15:40/15:43/15:44/15:47/15:49/15:50/15:53/15:54/15:56/15:57/15:60/15:62/15:63/15:64/15:65/15:66/15:67/15:71/15:74/15:75/15:79N/15:80/15:81/15:82/15:87/15:90/15:92/15:93/15:94N/15:95/15:96/15:97/15:98/15:99/15:102/15:103/15:104/15:105/15:110/15:113/15:115/15:117/15:118/15:119/15:120/15:122/15:125/15:127/15:128/15:129/15:132/15:133/15:134/15:135/15:137/15:138/15:140/15:142/15:145/15:146/15:147/15:148/15:152/15:153/15:156/15:157/15:158/15:159/15:160/15:163/15:164/15:165/15:166/15:167/15:171/15:172/15:173/15:174/15:175/15:176/15:177/15:178/15:183/15:184/15:187/15:191/15:192/15:193/15:196/15:197/15:198/15:201/15:203/15:204/15:205/15:206/15:208/15:210/15:211/15:212/15:215/15:216/15:217/15:219/15:221	B*35:XX2 XX2:=:35:01/35:43/35:57/35:67/35:79/35:102	B*15:XX1 XX1:=:15:01/15:01N/15:20/15:102/15:104/15:140/15:146/15:183/15:187/15:190N/15:201/15:205/15:206/15:211/15:227/15:228/15:232/15:234	35:01:01G	15:01:01G
P08-1120	B35 B53	B*35:XX15 XX15:=:35:01/35:07/35:20/35:50/35:52/35:53N/35:54/35:57/35:64	B*53:XX16 XX16:=:53:01/53:03/53:05/53:09/53:10/53:12/53:13/53:15/53:18/53:20/53:21	B*35:CCUA CCUA:=:01/57	B*53:KJW KJW:=:01/25/26	35:01:01G	53:01:01

P08-2083	B35 B38	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*38:XX3 XX3:=:38:01/38:02/38:03/38:04/38:08/38:09/38:11/38:13/38:14/38:15/38:16/38:17/38:18/38:20/38:21/38:22/38:23/38:24/38:25/38:26/38:27	B*35:CCUA CCUA:=:01/57	B*38:REHH REHH:=:38:01/38:24/38:31/38:34N	35:01:01G	38:01:01G
P08-0663	B07 B35	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*07:XX1 XX1:=:07:02/07:03/07:04/07:10/07:111N/07:13/07:15/07:16/07:21/07:22/07:23/07:26/07:27/07:28/07:30/07:33/07:35/07:36/07:37/07:39/07:41/07:42/07:44/07:45/07:46/07:47/07:49N/07:50/07:51/07:52/07:54/07:55/07:56/07:57/07:58/07:59/07:61/07:62/07:63/07:66/07:67N/07:70/07:71/07:73/07:74/07:75/07:76/07:79/07:81/07:82/07:83/07:85/07:87/07:88/07:89/07:91/07:92/07:93/07:94/07:96/07:98/07:99/07:101/07:102/07:103/07:104/07:106/07:107/07:108/07:109/07:113/07:114/07:115/07:116/07:117/07:118/07:119/07:120/07:121/07:122/07:124	B*35:CCUA CCUA:=:01/57	B*07:RDFP RDFP:=:07:02/07:44/07:49N/07:58/07:59/07:61/07:67N/07:101/07:103/07:104/07:106/07:108/07:111N/07:119/07:120/07:121/07:126/07:128/07:129/07:130/07:132/07:136/07:141/07:142/07:144	35:01:01G	07:02:01G
P08-0403	B35 B15	B*35:XX4 XX4:=:35:01/35:11/35:134N/35:130N/35:42/35:43/35:50/35:52/35:53N/35:54/35:57/35:64/35:67/35:68/35:79/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:119/35:120/35:122/35:123/35:126/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148/35:159	B*15:XX3 XX3:=:15:01N/15:01/15:03/15:04/15:08/15:11/15:12/15:149N/15:15/15:16/15:17/15:182N/15:181N/15:18/15:19/15:190N/15:209N/15:20/15:21/15:23/15:24/15:25/15:26N/15:28/15:32/15:34/15:35/15:36/15:38/15:39/15:40/15:43/15:44/15:47/15:49/15:50/15:52/15:53/15:54/15:56/15:57/15:60/15:62/15:64/15:65/15:66/15:67/15:71/15:74/15:75/15:79N/15:80/15:81/15:82/15:87/15:92/15:93/15:94N/15:95/15:96/15:97/15:98/15:102/15:103/15:104/15:105/15:110/15:113/15:114/15:115/15:117/15:118/15:119/15:120/15:122/15:124/15:125/15:127/15:128/15:129/15:132/15:133/15:134/15:135/15:137/15:138/15:140/15:142/15:145/15:146/15:147/15:148/15:152/15:153/15:156/15:157/15:158/15:159/15:160/15:163/15:164/15:165/15:166/15:167/15:171/15:172/15:173/15:174/15:175/15:176/15:177/15:178/15:183/15:184/15:187/15:191/15:192/15:193/15:196/15:197/15:198/15:201/15:203/15:204/15:205/15:206/15:208/15:210/15:211/15:212/15:215/15:216/15:217/15:219/15:221	B*35:XX2 XX2:=:35:01/35:43/35:57/35:67/35:79/35:102	B*15:XX1 XX1:=:15:01/15:01N/15:20/15:102/15:104/15:140/15:146/15:183/15:187/15:190N/15:201/15:205/15:206/15:211/15:227/15:228/15:232/15:234	35:01:01G	15:01:01G
P08-0172	B35 B39	B*35:XX4 XX4:=:35:01/35:03/35:49/35:50/35:52/35:54/35:55/35:57/35:70	B*39:XX5 XX5:=:39:01L/39:01/39:02/39:05/39:07/39:09/39:10/39:13/39:15/39:16/39:19/39:20/39:22/39:	B*35:CCUA CCUA:=:01/57	B*39:REHU REHU:=:39:01/39:01L/39:05/39:46/39:59/39:67	35:01:01G	39:01:01G

			23/39:25N/39:26/39:27/39:28/39:31/39:38Q/39:39/39:40N/39:41/39:42/39:44/39:45/39:46/39:47/39:48/39:49/39:51/39:52/39:53/39:54/39:55/39:56/39:59/39:61				
P08-0644	B35 B41	B*35:XX5 XX5:=:35:01/35:49/35:50/35:52/35:54/35:57	B*41:XX6 XX6:=:41:02/41:11/41:15	**ABMIGUOUS** **SSP not clear** B*35:RSXN B*35:63 RSXN:=:35:01/35:29/35:40N/35:42/35:57/35:94/35:101/35:108/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:144/35:145N/35:147/35:161/35:165N/35:168/35:170/35:173N/35:177	B*41:02 B*35:87	35:01:01G	41:02:01
P08-0111	B07 B35	B*35:08	B*07:XX1 XX1:=:07:02/07:03/07:04/07:10/07:111N/07:13/07:15/07:16/07:21/07:22/07:23/07:26/07:27/07:28/07:30/07:33/07:35/07:36/07:37/07:39/07:41/07:42/07:44/07:45/07:46/07:47/07:49N/07:50/07:51/07:52/07:54/07:55/07:56/07:57/07:58/07:59/07:61/07:62/07:63/07:66/07:67N/07:70/07:71/07:73/07:74/07:75/07:76/07:79/07:81/07:82/07:83/07:85/07:87/07:88/07:89/07:91/07:92/07:93/07:94/07:96/07:98/07:99/07:101/07:102/07:103/07:104/07:106/07:107/07:108/07:109/07:113/07:114/07:115/07:116/07:117/07:118/07:119/07:120/07:121/07:122/07:124	B*35:08	B*07:RDFP RDFP:=:07:02/07:44/07:49N/07:58/07:59/07:61/07:67N/07:101/07:103/07:104/07:106/07:108/07:111N/07:119/07:120/07:121/07:126/07:128/07:129/07:130/07:132/07:136/07:141/07:142/07:144	35:08:01	07:02:01G
P08-0018	B18 B35	B*35:XX5 XX5:=:35:03/35:55/35:70	B*18:XX4 XX4:=:18:01/18:02/18:03/18:04/18:05/18:06/18:08/18:09/18:13/18:17N/18:18/18:20/18:23N/18:24/18:25/18:27/18:28/18:29/18:31/18:32/18:33/18:34/18:37/18:38/18:39/18:40/18:41/18:42/18:43/18:44/18:45/18:46/18:47/18:50/18:51/18:52/18:53/18:54/18:55/18:59	B*35:CGAH CGAH:=:03/70	B*18:RBDJ RBDJ:=:01/53/55/59/62/63	35:03:01G	18:01:01G
P08-0737	B35	Taq issue		**SSP NOT CLEAR**		35:01:01G	44:03:01G

	B41			B*35:REFK REFK:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:108/35:116/35:119/35:120/35:122/35:123/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*44:MDBH MDBH:=:02/03/114/115/122/125		
P08-0756	B35 B44	B*35:XX10 XX10:=:35:01/35:130N/35:134N/35:42/35:48/35:50/35:52/35:54/35:57/35:68/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*44:XX11 XX11:=:44:03/44:07/44:108N/44:13/44:26/44:29/44:30/44:32/44:35/44:36/44:37/44:38/44:39/44:40/44:41/44:46/44:50/44:57/44:60/44:61N/44:65/44:69/44:81/44:85/44:92/44:94/44:96/44:98/44:99/44:103/44:105/44:109/44:111/44:114/44:115/44:120/44:122/44:125	B*35:CCUA CCUA:=:01/57	B*44:MDBK MDBK:=:03/114/115/122/125	35:01:01G	44:03:01G
P08-0645	B35 B5701	B*35:XX9 XX9:=:35:53N/35:64	B*57:XX10 XX10:=:57:01/57:06/57:08/57:10/57:11/57:15/57:16/57:18/57:20/57:21/57:23/57:27/57:29/57:31/57:32/57:33/57:34/57:35/57:36/57:37/57:38/57:40/57:41	B*35:CCUA CCUA:=:01/57	B*57:RBJP RBJP:=:01/29/33/35/36/37/41/44/47/48/52	35:01:01G	57:01:01G
P08-1048	B35 B44	B*35:XX1 XX1:=:35:01/35:130N/35:134N/35:41/35:42/35:48/35:50/35:52/35:54/35:57/35:68/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*44:XX2 XX2:=:44:02S/44:02/44:06/44:08/44:09/44:11/44:19N/44:22/44:23N/44:24/44:27/44:33/44:34/44:44/44:48/44:49/44:52N/44:53/44:55/44:59/44:63/44:66/44:67/44:68/44:71/44:72/44:73/44:74/44:80/44:84/44:86/44:87/44:88/44:89/44:91/44:93/44:95/44:101/44:102/44:104/44:112/44:113/44:116/44:117/44:118/44:119/44:121/44:126/44:127	B*35:CCUA CCUA:=:01/57	B*44:XX1 XX1:=:44:02/44:02S/44:27/44:66/44:104/44:118/44:119/44:121/44:126/44:137	Not enough sample for sequencing	
P08-0693	B35	B*35:XX9 XX9:=:35:01/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94	B*35:XX10 XX10:=:35:01/35:134N/35:130N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/3	B*35:XX1 XX1:=:35:01/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131	B*35:XX2 XX2:=:35:01/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:121/35:122/35:123/35:12	Not enough sample for sequencing	

			5:119/35:120/35:121/35:122/35:123/35:126/35:131/35:132/35:133/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	6/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177		
P08-1877	B27 B35	B*35:XX8 XX8:=:35:01/35:50/35:52/35:53N/35:54/35:57	B*27:XX7 XX7:=:27:14/27:23/27:41	B*35:CCUA CCUA:=:01/57	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84	Not enough sample for sequencing	
P08-1308	B35 B62	B*35:XX4 XX4:=:35:01/35:11/35:130N/35:134N/35:42/35:43/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:67/35:68/35:71/35:79/35:86/35:90/35:91/35:92/35:93/35:94/35:99/35:101/35:102/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:117/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*15:XX3 XX3:=:15:01N/15:01/15:03/15:04/15:08/15:11/15:12/15:149N/15:14/15:15/15:16/15:17/15:18/15:182N/15:181N/15:190N/15:19/15:209N/15:20/15:21/15:23/15:24/15:25/15:26N/15:28/15:32/15:34/15:35/15:36/15:38/15:39/15:40/15:43/15:44/15:47/15:49/15:50/15:53/15:54/15:56/15:57/15:60/15:62/15:64/15:65/15:66/15:67/15:71/15:74/15:75/15:79N/15:80/15:81/15:82/15:87/15:92/15:93/15:94N/15:95/15:96/15:97/15:98/15:102/15:103/15:104/15:105/15:110/15:113/15:115/15:117/15:118/15:119/15:120/15:122/15:125/15:127/15:128/15:129/15:131/15:132/15:133/15:134/15:135/15:137/15:138/15:140/15:142/15:145/15:146/15:147/15:148/15:152/15:153/15:156/15:157/15:158/15:159/15:160/15:161/15:163/15:164/15:165/15:166/15:167/15:171/15:172/15:173/15:174/15:175/15:176/15:177/15:178/15:183/15:184/15:187/15:191/15:192/15:193/15:196/15:197/15:198/15:201/15:203/15:204/15:205/15:206/15:208/15:210/15:211/15:212/15:215/15:216/15:217/15:219/15:221	**SSP NOT CLEAR** B*35:RJVZ RJVZ:=:35:01/35:14/35:29/35:32/35:40N/35:42/35:43/35:57/35:67/35:68/35:79/35:94/35:101/35:102/35:107/35:108/35:116/35:119/35:120/35:121/35:122/35:123/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:144/35:147/35:148/35:161/35:165N/35:168/35:170/35:173N/35:177	B*15:RJTJ RJTJ:=:15:01/15:01N/15:05/15:20/15:70/15:78/15:85/15:102/15:104/15:140/15:146/15:154/15:165/15:183/15:187/15:190N/15:201/15:205/15:206/15:211/15:227/15:228/15:232/15:234	35:01:01G	15:01:01G
P08-1943	B27 B35	B*35:XX7 XX7:=:35:03/35:55/35:70	B*27:XX6 XX6:=:27:14/27:23	**SSP NOT CLEAR** B*35:REGN REGN:=:03/70/106/127/152/160/181	B*27:REDU REDU:=:27:05/27:13/27:64N/27:72/27:84	35:03:01G	27:05:02G
P08-0584	B35 B58	Plate not readable		**SSP NOT CLEAR**		35:01:01G	58:02:00

				B*35:REEX REEX:=:35:01/35:40N/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:116/35:119/35:120/35:121/35:122/35:123/35:126/35:130N/35:131/35:132/35:133/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	B*58:02		
P08-1760	B07 B35	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*07:XX1 XX1:=:07:02/07:03/07:04/07:10/07:111N/07:13/07:15/07:16/07:21/07:22/07:23/07:26/07:27/07:28/07:30/07:33/07:35/07:36/07:37/07:39/07:41/07:42/07:44/07:45/07:46/07:47/07:49N/07:50/07:51/07:52/07:54/07:55/07:56/07:57/07:58/07:59/07:61/07:62/07:63/07:66/07:67N/07:70/07:71/07:73/07:74/07:75/07:76/07:79/07:81/07:82/07:83/07:85/07:87/07:88/07:89/07:91/07:92/07:93/07:94/07:96/07:98/07:99/07:101/07:102/07:103/07:104/07:106/07:107/07:108/07:109/07:113/07:114/07:115/07:116/07:117/07:118/07:119/07:120/07:121/07:122/07:124	B*35:CCUA CCUA:=:01/57	B*07:RDFP RDFP:=:07:02/07:44/07:49N/07:58/07:59/07:61/07:67N/07:101/07:103/07:104/07:106/07:108/07:111N/07:119/07:120/07:121/07:126/07:128/07:129/07:130/07:132/07:136/07:141/07:142/07:144	35:01:01G	07:02:01G
P08-0088	B07 B35	B*35:XX2 XX2:=:35:03/35:55/35:70	B*07:XX1 XX1:=:07:02/07:03/07:04/07:10/07:111N/07:13/07:15/07:16/07:21/07:22/07:23/07:26/07:27/07:28/07:30/07:33/07:35/07:36/07:37/07:39/07:41/07:42/07:44/07:45/07:46/07:47/07:49N/07:50/07:51/07:52/07:54/07:55/07:56/07:57/07:58/07:59/07:61/07:62/07:63/07:66/07:67N/07:70/07:71/07:73/07:74/07:75/07:76/07:79/07:81/07:82/07:83/07:85/07:87/07:88/07:89/07:91/07:92/07:93/07:94/07:96/07:98/07:99/07:101/07:102/07:103/07:104/07:106/07:107/07:108/07:109/07:113/07:114/07:115/07:116/07:117/07:118/07:119/07:120/07:121/07:122/07:124	B*35:CGAH CGAH:=:03/70	B*07:RDFP RDFP:=:07:02/07:44/07:49N/07:58/07:59/07:61/07:67N/07:101/07:103/07:104/07:106/07:108/07:111N/07:119/07:120/07:121/07:126/07:128/07:129/07:130/07:132/07:136/07:141/07:142/07:144	35:03:01G	07:02:01G
P08-0656	B35 B47	B*35:XX7 XX7:=:35:01/35:23/35:50/35:52/35:54/35:57	B*47:XX8 XX8:=:47:01/47:02/47:03/47:06/47:07/47:08	B*35:XX1 XX1:=:35:01/35:23/35:57	B*47:BC BC:=:02/03	35:01:01G	47:03

P08-1224	B35 B14	B*35:XX2 XX2:=:35:01/35:50/35:52/35:54/35:57	B*14:XX1 XX1:=:14:02/14:03/14:04/14:06/14:09/14:15/14:16/14:17/14:18/14:20	B*35:CCUA CCUA:=:01/57	B*14:EGG EGG:=:02/22	35:01:01G	14:02:01
P08-0688	B35 B51	B*35:XX1 XX1:=:35:03/35:55/35:70	B*51:XX2 XX2:=:51:01/51:02/51:03/51:07/51:11N/51:12/51:14/51:18/51:22/51:26/51:27N/51:28/51:30/51:32/51:33/51:35/51:38/51:39/51:41N/51:43/51:48/51:50/51:51/51:52/51:53/51:57/51:58/51:60/51:65/51:66/51:67/51:68/51:69/51:70/51:71/51:75/51:76/51:77/51:78/51:79/51:83/51:84/51:88/51:89/51:94/51:96/51:98N/51:99/51:100/51:102/51:103/51:104/51:105	B*35:CGAH CGAH:=:03/70	B*51:XX1 XX1:=:109/117/123	35:03:01G	51:01:01G
P08-1331	B3502 B35	B*35:XX2 XX2:=:35:01/35:04/35:130N/35:134N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:132/35:137/35:138/35:139/35:140/35:143/35:144/35:147/35:148	B*35:XX3 XX3:=:35:02/35:129N/35:146	B*35:RMGE RMGE:=:35:02/35:129N/35:146/35:182/35:183	B*35:XX1 XX1:=:35:01/35:04/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:130N/35:132/35:134N/35:137/35:138/35:139/35:140/35:144/35:147/35:148/35:161/35:165N/35:168/35:170/35:173N/35:177	35:01:01G	35:02:01
P08-0163	B35 B35	B*35:XX8 XX8:=:35:01/35:130N/35:134N/35:145N/35:42/35:49/35:50/35:52/35:53N/35:54/35:57/35:63/35:64/35:68/35:77/35:78/35:82/35:90/35:91/35:92/35:93/35:94/35:101/35:103/35:104/35:107/35:108/35:110/35:111/35:112/35:116/35:118/35:119/35:120/35:122/35:123/35:126/35:131/35:132/35:137/35:138/35:139/35:140/35:141/35:143/35:144/35:147/35:148/35:159	B*35:XX9 XX9:=:35:03/35:55/35:65Q/35:70/35:74/35:75/35:84/35:85/35:98/35:106/35:127/35:128/35:150/35:151/35:152/35:153/35:155/35:160	**SSP NOT CLEAR** B*35:RJWG RJWG:=:35:01/35:29/35:40N/35:42/35:57/35:94/35:101/35:103/35:107/35:108/35:111/35:112/35:116/35:119/35:120/35:122/35:123/35:126/35:130N/35:131/35:132/35:134N/35:137/35:138/35:139/35:140/35:141/35:144/35:145N/35:147/35:148/35:159/35:161/35:165N/35:166/35:168/35:170/35:173N/35:177	 B*35:RJXB RJXB:=:03/70/106/127/128/136/152/153/155/160/179/181	35:01:01G	35:03:01G