RECENT HIV SEROCONVERSION AT TIME OF FIRST POSITIVE TEST: A COMPARISON BEFORE AND AFTER HIV REPORTABILITY

by

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ABSTRACT

Background:

HIV was added to the British Columbia list of reportable diseases on 1 May 2003 which included enhanced contact tracing by public health. A sensitive/less-sensitive (S/LS) algorithm using a modified EIA anti-HIV assay was employed to evaluate enhanced partner notification by comparing the proportion of newly diagnosed cases of HIV presenting within 6 months of becoming infected before and after HIV Reporting.

Methods:

Banked HIV positive samples, collected between 1 Jan 2000– 30 Apr 2003 (pre-reporting group) and 1 May 2003 – 23 Aug 2006 (post-reporting group) were re-tested using the bioMérieux Vironostika HIV-1-S/LS tests. Samples were classified by the S/LS EIA (detuned test) as a recent seroconversion (RSC) (infected for <170 days) or established infection (>170 days). Data was linked to the BC HIV Surveillance and AIDS databases. The proportion of RSC in the pre-reporting group was compared to the proportion of RSC in the post-reporting group using a 2-sided z-test of independent proportions. Similarly, the proportion of new cases of HIV presenting with AIDS was compared between groups. A Kappa statistic was calculated to determine the level of agreement between clinical assessment of HIV staging was compared and the detuned test results. Finally, characteristics of RSC were examined.

Results:

Serum was available for 1111 newly positive HIV cases in the pre-reporting group and 470 in the post-reporting group. RSC in the pre and post reporting group were 311 (28%; CI: 25.36%, 30.73%) and 136 (29%; CI: 24.87%, 33.27%) respectively (p= 0.70). There was no significant difference in the proportion of cases presenting with AIDS between groups (pre-reporting: 6.7%

[CI: 5.4%, 8.1%]; post-reporting: 7.6% [CI: 6.3%, 9.1%]) (p=0.31). Sex work is independently associated with being RSC (AOR 1.78 [CI:1.09, 2.91]). There is an inverse association between being 41-60 yrs old, Asian and/or mixed ethnicity and RSC.

Conclusions:

The bioMérieux Vironostika HIV-1-S/LS test is an effective tool to objectively evaluate public health interventions and in identifying sub-populations likely to be RSC. This underpowered study demonstrated a slight increase in RSC post reporting which was not statistically significant. Similarly there was no difference in the proportion of cases presenting with AIDS.

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ACRONYMS

AI	Avidity Index
AIDS	Acquired Immune Deficiency Syndrome
ARV	Antiretroviral
BC	British Columbia
CRF	Case Report Form
DNA	Deoxyribonucleic acid
EIA	Enzyme Immunoassay
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HIVSS	HIV Surveillance System
HSDA	Health Service Delivery Area
IDU	Injection Drug Use(rs)
LR	Likelihood Ratio
LSC	Late Seroconvertors (also referred to as Established Infections)
MSM	Men who have Sex with Men
NAAT	Nucleic Acid Amplification Testing
RNA	Ribonucleic acid
RSC	Recent Seroconversion
S/CO	Sample to Cut-off ratio
S/LS	Sensitive Less-Sensitive test
SOD	Standard Optical Density

STI	Sexually Transmitted Infection
STW	Sex Trade Worker
WB	Western Blot

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DEDICATION

I would like to dedicate this thesis to my daughter, Kate Thomson, for her never ending patience with having a mother who is also a student and for sacrificing many hours of family time to allow me to complete this leg of my education.

CHAPTER 1: BACKGROUND

1.1 Burden of Disease

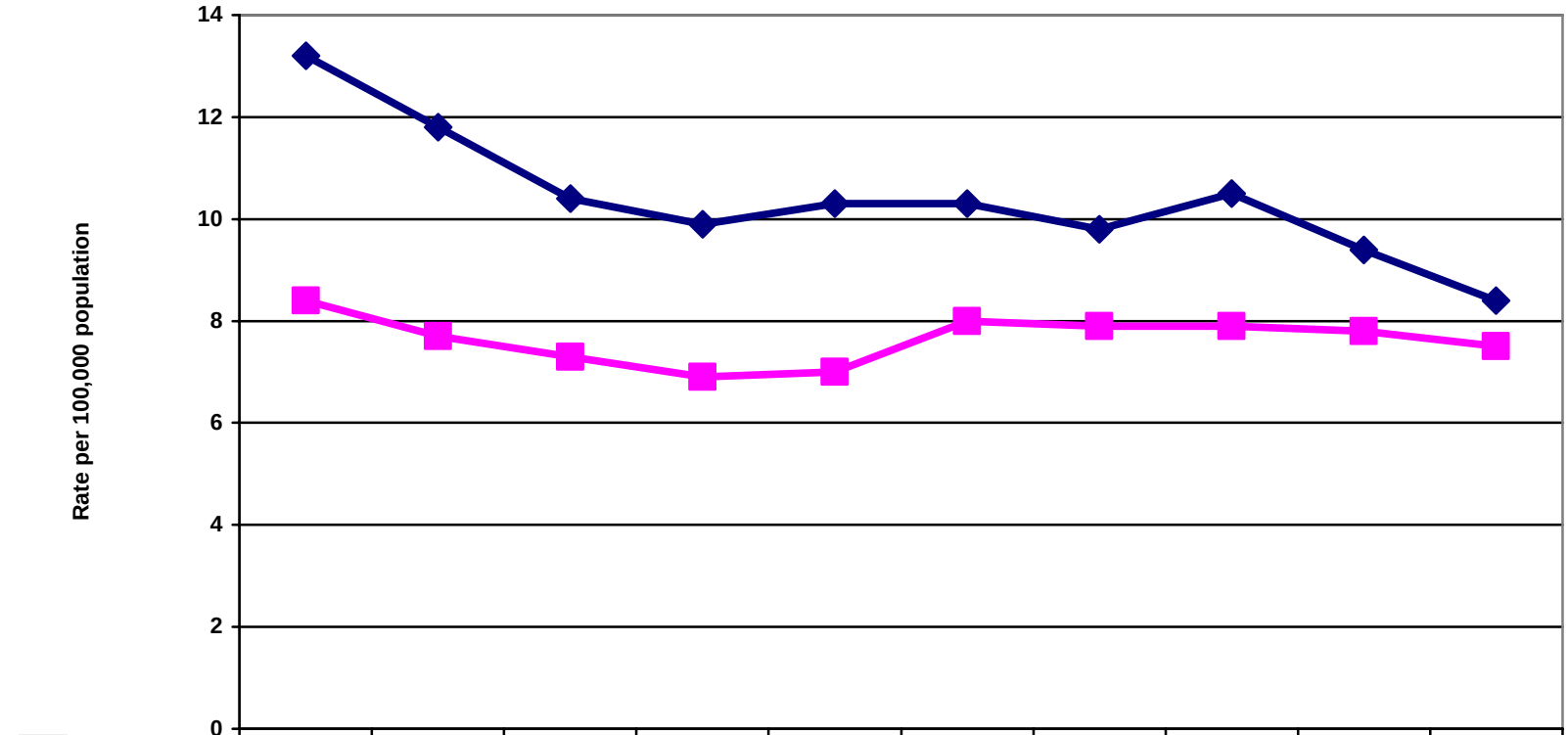
Human Immunodeficiency Virus (HIV), is a retrovirus that attacks the human immune system and leads to AIDS (1). This rapidly replicating virus is blood-borne and is spread by sexual intercourse, intravenous drug use, vertical transmission to fetuses in pregnancy, unscreened blood transfusion, organ transplantation or occupational exposure to HIV contaminated blood or body fluids (1).

1.1.1 Epidemiology of HIV/AIDS

Approximately 2518 Canadians had newly documented HIV infection reported in 2005 (2). While a small number of these cases (n=27) occurred in children (< 15 yrs old), the remainder occurred in adults.(2) British Columbia (BC) ranks third in the number of newly reported cases in Canada with 20.4% of Canadian 2005 cases in BC. Of more concern is that BC's annual rate is highest in Canada (9.4/100,000 population compared to 9.2/100,000 in Ontario and 7.5/100,000 in Quebec in 2005) (2-3). Although the annual rate in BC has been higher than our national rates (at least since 1997), the rate differential has narrowed over the past 10 years as seen in the following graph (Figure 1.1) (3).

While HIV is found throughout BC, the majority of cases are reported in Vancouver (44% of all cases) and the annual rate per 100,000 is significantly higher in Vancouver (32.7/100,000) compared to other health service delivery areas (HSDA) in BC. The next highest is Northern Interior HSDA with a rate of 14.3 in 2006. HIV in BC is a disease mainly found in men with 80% of new reports are related men[3] This is slightly higher than the national statistic of 74% cases found among men (2).

Figure 1.1 Annual Rate of Newly Reported Cases of HIV in British Columbia and Canada



	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
BC Reports	521	471	416	401	421	422	409	442	401	363
BC Rate	13.2	11.8	10.4	9.9	10.3	10.3	9.8	10.5	9.4	8.4
Canadian Rate	8.4	7.7	7.3	6.9	7	8	7.9	7.9	7.8	7.5

The proportion of cases reported annually in BC is currently highest among men who have sex with men (MSM) (40.5%) and intravenous drug users (IDU) (26.2%). The remaining number of cases is found among heterosexuals, non-IDU individuals and individuals with other risk (i.e. occupational exposure). In addition to the 363 new cases in BC in 2006, 51 were from individuals who contracted HIV outside BC.

According to the BC AIDS surveillance database, there were 102 new reports of AIDS in BC during 2005 which equates to an annual rate of 2.5 per 100,000 population. The gender breakdown of these cases is comparable to gender breakdown of HIV with 81% of AIDS cases being in men with the greatest proportion aged 30-60 years old.

Although the number of new infections per year in Canada has remained fairly constant over recent years, the rate of reporting of new positive HIV tests in British Columbia increased slightly. from 10.1/100,000 in 2003 to 10.9/100,000 in 2004 and then decreased again to 9.9 in 2005 (4).

Estimates of HIV prevalence in Canada suggest that there were 58,000 people living with HIV and AIDS at the end of 2005 (5). Of these, approximately 15,800 (27%) were not aware of their infection (5). This has been termed the 'hidden epidemic'. It is likely that some of the hidden epidemic consists of newly positive people who tested for HIV prior to the time that a standard test produces a positive result (i.e. during the window period). These people will be given a negative result leaving them with an erroneous impression of their HIV status, while remaining at high risk for transmitting to others.

1.2 The Virus

HIV has been studied with great interest since it was first recognized in 1981. HIV is an enveloped virus. The envelope contains viral glycoproteins such as gp120 and gp41. The core of the virus contains the p24 and p17 proteins. Also inside the core are two copies of single-stranded RNA viral genome which are accompanied by reverse transcriptase, protease and integrase. These enzymes play an important role in the replication of the virus. When infection occurs, the virus attaches to the host cell (i.e. a T-lymphocyte helper cell) through an interaction between gp120 (on the virus) and the CD4 antigen receptor on the host cell. After the attachment occurs, the virus and host cell membranes fuse allowing the virus to enter into the host's cell through a nuclear pore. The viral RNA then moves into the cytoplasm of the host cell, and the reverse transcriptase makes two DNA copies of the viral RNA genome. At the same time the viral RNA is degraded. The ends of the double stranded DNA join forming a circular formation. This circular formation moves to the nucleus of the host cell and is inserted into the host cell chromosome. This integrated DNA is what's referred to as a proviral DNA. When the host's cell is stimulated, viral RNA is synthesized from the proviral DNA . The new RNA leaves the host cell, through a nuclear pore. Some of the viral RNA then forms a new viral genome and some of the RNA produce new viral proteins. Together, these components assemble a new HIV cell (6).

The lifespan of the virus is relatively short (half-life equals 6 hours) but it replicates very rapidly. It has been determined that the replication rate of HIV is approximately 10 billion viral particles per day (7). Progressive loss of CD4 cells results in immunodeficiency and susceptibility to opportunistic infections which eventually kills the host.

1.3 The Host's Response to HIV

Shortly after infection, the host initiates an immunological response. Through a series of complex immunological events, antibodies specific to HIV are produced. These antibodies bind to antigen in the virus creating an antibody complex. Initially, antibody production is slower than HIV replication but gradually increases until it reaches a maximum level. This usually takes 3-6 months. In addition to the increasing rate of antibody production over time in early HIV illness, the strength with which the antibodies bind to the antigen (avidity) is weak early in HIV illness and gradually increases over time. Both the gradual production of HIV antibody and the gradual increase in avidity play an important role in differentiating early illness from established illness.

1.4 Natural History of HIV/AIDS

HIV infection progresses through an acute seroconversion phase, an early HIV infection phase, an asymptomatic phase (also known as clinical latency) and ultimately to full blown AIDS (8). Acute seroconversion refers to the stage from time of infection to approximately 6 weeks after infection. This phase is characterized by the lack of detectable antibodies making diagnosis, using current screening methodologies, difficult. During this phase, the virus is able to replicate in an uninhibited fashion causing the HIV viral levels in the plasma (also referred to as the viral load) to be very high. During the acute seroconversion phase, 30% of infected people experience influenza-like symptoms. This is referred to as acute seroconversion syndrome or viral syndrome (1). The gradual development of HIV antibodies and the body's cell mediated immune response causes the viral load to decline and at approximate 6 months post

infection the level of virus reaches a 'set point'. The set point refers to the level of viral load in blood which remains relatively stable during the clinical latency phase. Set points vary in HIV infected individuals and represent a balance between viral production and immune clearance. For the purposes of this study, the phase between infection and the 6 months following infection including early clinical latency is being termed recent seroconversion (RSC).

The progression of HIV from infection through to clinical latency has been described by Pantaleo et al.(9). During the first 3-6 weeks of infection (primary infection) plasma viremia occurs resulting in wide dissemination of the virus. During this time there is a rerouting of lymphocytes and patients may experience acute symptoms. During 1 week to 3 months following this, the body develops an immune response to HIV where there is a curtailment of plasma viremia and the virus is sequestered to the lymphoid tissue. Clinical latency occurs 1-2 weeks after this.

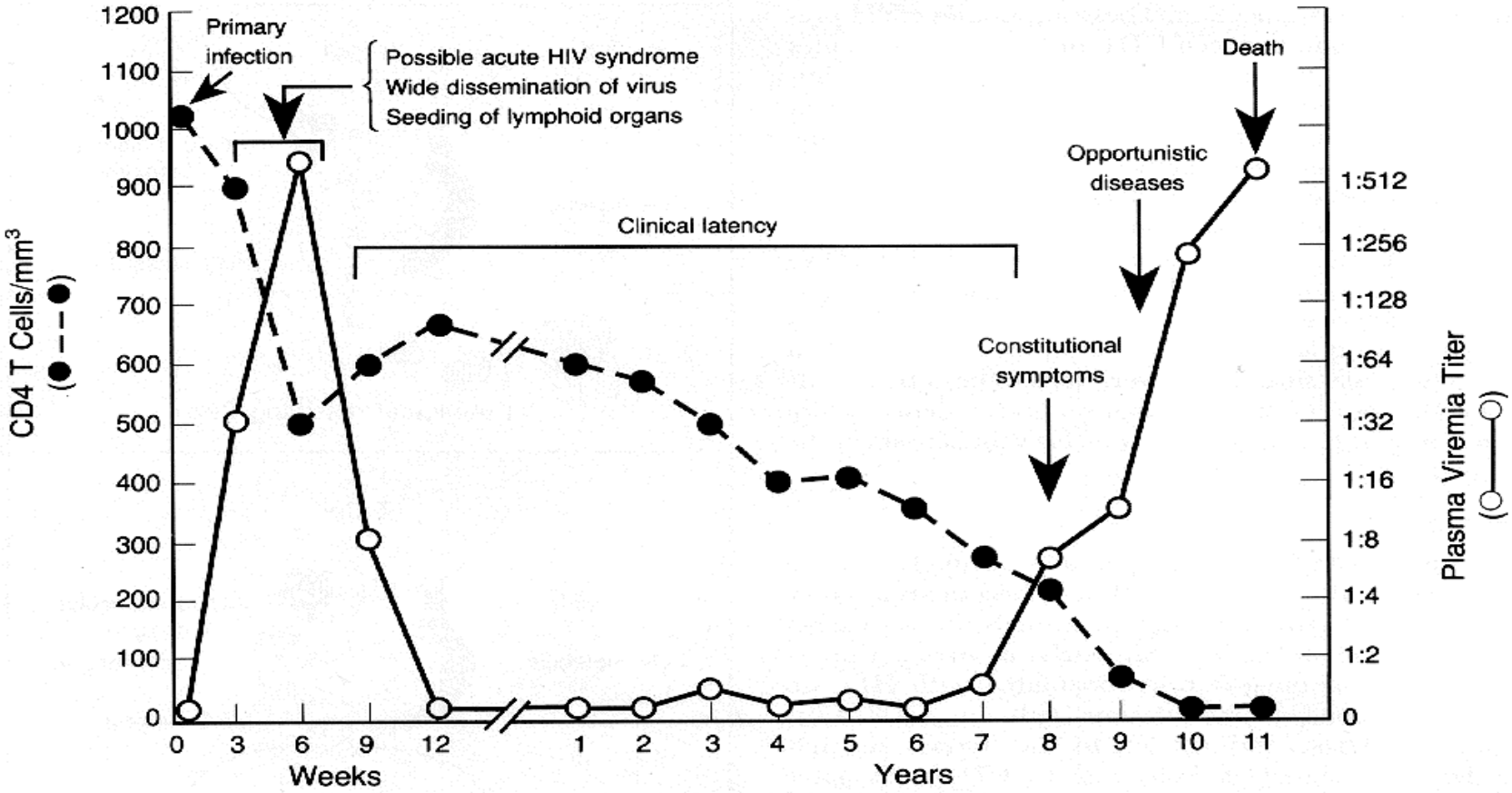
The length of the clinical latency phase also varies but lasts approximately 8-11 years (10). Eventually, the immune system begins to fail and HIV positive people enter a chronic infection phase when they become prone to opportunistic infections (i.e. thrush, herpes). Progressive immunosuppression leads to the development of full blown AIDS marked by AIDS defining illnesses. This list of AIDS defining illnesses in Canada is provided in Appendix B.

1.5 Relationship Between CD4 Counts and Viral Loads

There is an inverse relationship between CD4 cells and viral load (Figure 1.2). During early HIV illness, the amount of virus in the host's plasma is typically high making the host hyperinfectious to others. At that time, the number of CD4 cells is mildly reduced. Once antibody production reaches its maximum level seroconversion is said to have taken place and

the amount of virus in the plasma is reduced. The viral load stays at a relatively stable amount during clinical latency. Having said that, it should also be noted that numerous other factors influence the amount of circulating virus (i.e. stimulation of the immune system through introduction of a secondary infection such as syphilis). However, generally speaking, during this phase of HIV illness, a dynamic balance exists between CD4 cells and HIV virus as a result of replication, destruction, host compensation and virus suppression, thus keeping the CD4 cell count and viral load count stable. Late in HIV illness, the body's capacity to respond immunologically becomes reduced allowing the virus to replicate at a higher rate. Consequently, laboratory findings during this phase show low CD4 counts and high plasma viral load counts.

Figure 1.2 Inverse Relationship Between CD4 Counts and HIV Viral Load in Untreated Individuals (9)



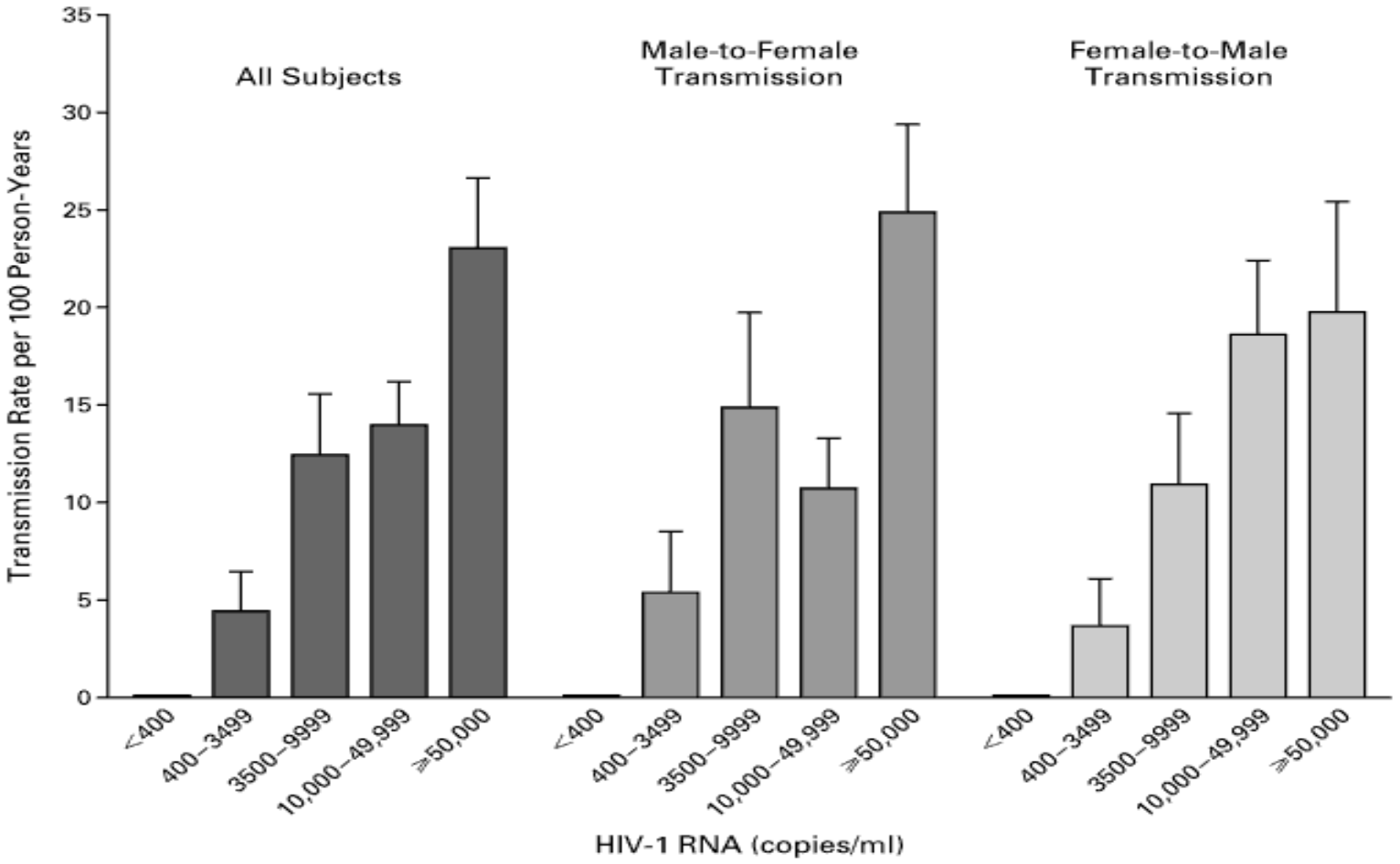
1.6 Modes of Transmission

HIV is a blood-borne pathogen that is transmitted through direct contact with mucous membranes containing bodily fluids harboring the virus such as blood, semen, vaginal fluid, and breast milk. It can also be transmitted vertically from mother to child during pregnancy and/or child birth. It can be transmitted through intercourse (vaginal or anal), oral sex, blood transfusions, and contaminated needles. Transmission is a function of the interaction between a sufficiently infectious HIV carrier, a susceptible host (11) and the environment. The term 'sufficiently infectious carrier' is particularly important as it speaks to the fact that the risk of secondary transmission is highest among carriers with high viral loads while the risk in carriers with non-detectable viral loads is very low. As the viral load increases in the infected person and the number of exposures increases in an individual, so does the risk of contracting the virus (12). Quinn and Wawer (12) were able to demonstrate, in their large Ugandan heterosexual cohort, that transmission is very unlikely to occur when serum viral load falls below 3000 copies. Conversely, during the first 5 months of infection risk of transmission can be as high as 1/25 – 1/1000 per coital act (12). Figure 1.3 shows the mean rate of heterosexual transmission according to serum HIV RNA levels.

1.7 Risk Factors

The terms 'susceptible carrier' and 'environment' mentioned above refers to the social and behavioural risk factors associated with disease transmission. There are greater proportions of HIV acquisition among marginalized populations, such as MSM, sex workers, and street involved/homeless populations (4). In addition, it was been previously shown that episodes of co-infection with other sexually transmitted infections

Figure1.3 Mean Rate of Heterosexual Transmission According to Serum HIV RNA Levels (12)



increase viral loads in genital secretions (11). Research has shown that people with genital ulcer disease, such as HSV-2, are at twice the risk of contracting HIV (13).

Unprotected sexual activity, sex with persons known to be HIV positive, sex with multiple partners, anal intercourse and needle sharing are all considered high risk behaviours related to HIV acquisition (10). The relative risk for insertive and receptive fellatio is thought to be quite low (RR:1-2 respectively), and insertive intercourse moderately low (RR vaginal: 10; RR anal: 13). Receptive intercourse, particularly receptive anal intercourse is significantly higher with a relative risk of 100 (14).

1.8 Diagnosis

In British Columbian adults, HIV is currently diagnosed using serological antibody tests such as enzyme immunoassay (EIA) and Western Blot. Typically, positive EIA tests are confirmed using a Western Blot antibody test. Under rare circumstances, HIV infection is diagnosed with antigen tests (such as p24 tests) or nucleic acid amplification tests (described in chapter two). These tests have the capability of detecting HIV before antibody development.

From a clinical perspective, a diagnosis of HIV during the acute seroconversion window, (also referred to as acute seroconversion, preseroconversion, or primary infection) is unlikely to be made unless nucleic acid testing is performed. This is because during the preseroconversion window period HIV antibody is below the threshold level of assay detection (15). However, detection of acute HIV after seroconversion is feasible because anti-HIV assays can be 'detuned' to detect and differentiate the antibody concentration associated with new infection from the relatively strong antibody responses associated with chronic infection. This can be accomplished by re-testing HIV positive specimens with an EIA test that is less sensitive (LS) than the standard

EIA test. Similar tests are available to test the avidity (strength) of the antibody complex (see Chapter 2 for more details).

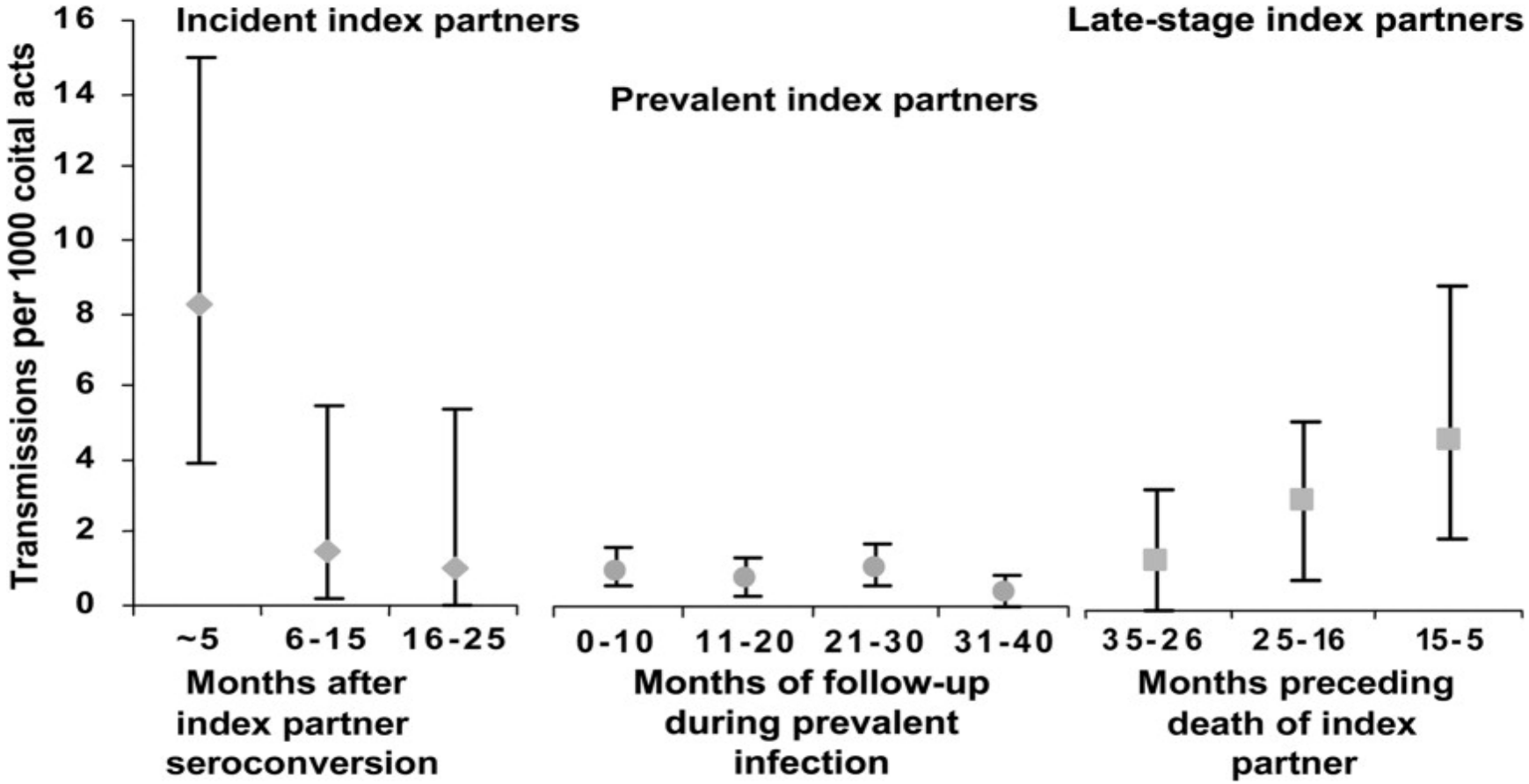
1.9 Importance of Early Detection

One reason why early detection is important is that identification of seroincident cases provides data to determine estimates of true incidence and prevalence in populations. For example, Boulos et al. (16) were able to calculate estimates of the incidence and prevalence of HIV in Canada for 2005 using a calculation involving the proportion of recently infected cases.

An important reason for detecting an HIV infection is to capitalize on the opportunity to interrupt secondary transmission. As described, people newly infected (i.e. in the first 3-6 months of infection) have a higher viral load and a greater probability of transmission than those at other stages of disease. The Imperial College group claim that people who are acutely infected are 28 times more infectious than during clinical latency phase (17). The proportion of recently infected individuals in a population impacts the growth of an epidemic, especially if these seroincident cases are unaware of their HIV status. Wawer et al. (18) found that 43.2% of all transmission events in their cohort could be ascribed to index (infected) subjects with acute and early infection. However, this attributable risk is thought to be lower in populations where partner change and partnerships that occur at the same time (concurrency) is higher during clinical latency than the cohort studied by Wawer (18). The Figure 1.4 illustrates the rate of transmission per coital act in three different phases of HIV illness in a heterosexual epidemic.

Researchers have used secondary surveillance methods to determine the number of seroincident cases in their populations. For example, a report from Amsterdam revealed that among 454 MSM HIV cases, 37 (8% of all positives) were recent infections. Twenty-six (84%)

Figure 1.4 Rate of Transmission Per Coital Act in Three Different Phases of HIV Illness in a Heterosexual Epidemic (18)



of these recently infected men were not aware of their HIV positive status and reported engaging in high risk sexual activities such as high levels of partner concurrency and high rates of partner change(19). The overarching result of a systematic review by Marks et al.(20) stated that the prevalence of high risk sexual behaviour is, on average 53% (95% confidence interval CI: 45%-60%) lower in HIV positive persons aware of their status relative to those unaware of their status. This emphasizes the importance of diagnosing HIV during the stage when individuals are most infectious. This is consistent with BC findings among an IDU cohort which saw a reduction of 40% in self-reported needle sharing among HIV positive participants after learning of their HIV positive status (21). In summary, identification of seroincident cases enables targeted HIV prevention interventions and provides information on ongoing seroincidence rates which can be used to assess prevention programming.

1.10 Characteristics of Early Testers

With the availability of laboratory technology to detect recent seroconversions, it is possible to characterize individuals who are testing early in their HIV illness. This information informs public health practice, enabling increased frequency of testing in groups who may be at higher risk of both infections and transmitting to others. Previous researchers have found that being $\geq$ 36 years old (OR 2.5), having unprotected anal sex in the past 12 months (OR 2.98), and having a past history of gonnorhea (OR 3.03) were all associated with testing early rather than later in HIV illness among STD clinic attendees (22). Conversely, CDC Atlanta reported that persons testing newly positive late in their HIV illness were more likely to be heterosexual and individuals who have lower levels of education than early testers (23). There is some debate in the literature about which ethnic groups are most likely to be recently infected at the time of

diagnosis (22-23). Information about the characteristics of people who present early in their HIV illness at the time of diagnosis has helped informed public health decisions when designing interventions aimed at reducing barriers to early diagnosis of HIV (23-24).

1.11 Effects of Post Test Counselling

As mentioned above, informing individuals of their HIV positive status, as in the context of post-test counselling, has an effect of risk behaviour. Otten et al. (25) reported that, among their urban STD clinic study population, there was a 12% decrease in acquisition of STIs with post test counselling compared to a 103% increase in STIs among the HIV negative clients after counselling. The European Partner Notification Study Group reported that 18-29% of previously unknown HIV infections can be uncovered through partner notification efforts and a greater proportion of partners were HIV positive in cases that had recently seroconverted (38% vs 27%) (26). These data support enhanced contact tracing initiatives associated with making HIV a reportable disease.

1.12 HIV Reporting

On 1 May, 2003 the British Columbia *Health Act* was amended to add HIV to the list of reportable diseases in the province (27). It was assumed that enhanced partner notification, as conducted in the HIV Reporting process, would result in prompt partner notification, early screening and early diagnosis of positive cases. However, a community consultation conducted by the Provincial Health Officer (PHO) revealed lack of support for making HIV reporting out of fear of the potential for breaches in confidentiality with public health involvement. This might

result in individuals delaying getting tested (28). There is controversy in the literature about whether mandatory named-based reporting or policy influences testing behavior.

Early research reports reveal that, in general, testing behaviour is not significantly influenced by reporting policy (28-30). Policy makers have made attempts at avoiding delays in testing by offering non-nominal or anonymous testing. Non-nominal testing refers to HIV testing that is ordered using a code or the initials of a person being tested. In this circumstance, the testing physician/care giver knows the identity of the tester but positive test results are reported to public health using the code or initials the person used at the time of testing. In anonymous testing the physician/care provider does not know the testers true identity and the test result is reported using a code that was assigned at the time of testing (31). These testing methods provide a level of confidentiality that some clients prefer. However, some reports in the literature suggesting that high risk groups who prefer anonymous testing are likely to avoid testing when mandatory reporting is legislated even when offered the choice of non-nominal testing (32). A recent report from the US Centers for Disease Control confirms that the concerns voiced surrounding mandatory reporting and partner notification have diminished over time (33). Although some reports of delays in testing exist, it has been suggested that such delays are most likely due to apprehension about subsequent test results than concerns over public health involvement (34).

Stemming from the community consultation on making HIV reportable in BC, the Provincial Health Officer committed to conduct an evaluation of HIV Reporting two years after HIV was made reportable. The purpose of the evaluation was to evaluate the net benefits or harms of making HIV reportable (24). In the evaluation report, attempts were made to describe partner notification activities during post test counselling. However, due to lack of comparative

data, the evaluation was unable to provide quantitative evidence about the effectiveness of involving public health in partner notification in terms of reducing time from infection to diagnosis in partners.

Three systematic reviews, including one conducted by the Cochrane Collaboration, have been conducted summarizing the effects of partner notification on reducing onward transmission. Oxman et al.(35) reviewed 12 controlled studies and reported that partner notification conducted by a care provider (provider partner notification) was significantly more effective in finding new cases of HIV cases than when the index case notifies their own partners (index case notification) (risk difference of 0.43 [95% CI: 0.34, 0.52]). Later, Macke et al. (36) reviewed five HIV studies among a total of 14 studies involving partner notification for STIs. They concluded that partner notification conducted by health care providers yielded more partners being notified and medically evaluated than notification conducted by the index cases. The only comparative study included among the five HIV studies revealed that the proportion of partners elicited who were actually notified was 50% in the health care provider group compared to 7% in the index case group. More importantly, more infections in partners were identified in the health care provider group than in the index case group (25% and 20% respectively) (37). The final systematic review by Mathews et al., published in the Cochrane Library, reports similar findings (38).

While the evidence that partner notification conducted by health care providers consistently shows a positive effect on identification of new cases of HIV, the methods used to compare provider effects to index case effects are dependant on the index case's willingness to provide partner information. None of the studies used an objective method to determine if public health's involvement in partner notification was effective in reducing time from infection to diagnosis. Considering the resources invested in public health involvement in partner

notification, policy makers seek objective evidence to ensure that the resources applied to partner notification reduces the number of secondary cases.

1.13 Primary Study Objective

The primary objective of this study is to examine the association between enhanced contact tracing and being RSC at the time of HIV diagnosis pre and post HIV Reporting.

1.14 Secondary Objectives

1) To compare the proportion of individuals presenting with AIDS when first diagnosed with HIV pre and post HIV reporting.
2) To determine the degree of agreement in physician classification of RSC and a laboratory classification of RSC.
3) To examine the association between demographic/risk factors and being RSC at the time of HIV diagnosis.

1.15 Hypothesis:

There is $\geq$ 5% difference in the proportion of RSC among people testing newly positive prior to HIV Reporting compared to those testing newly positive post HIV Reporting. (H_O: P1 = P2; H_A: P1 - P2 $\geq$ 5% or P2 – P1 $\geq$ 5%; two-sided) where:

H_O = the null hypothesis

$H_{A:}$ = the alternative hypothesis

P1 = proportion of RSC in the pre reporting group

P2 = proportion of RSC in the post reporting group

CHAPTER 2: DESCRIPTION OF HIV TESTS

There are a number of commercially available laboratory tests for HIV. Some are designed to detect antibodies to HIV, some are designed to detect HIV antigens and some are designed to detect HIV's RNA or Proviral DNA. In addition, some tests are designed to differentiate people who are recently infected from those with more established disease. The period of time between infection and detection of virus through testing is considered the 'window period'. Although HIV tests have been done on saliva and urine, this chapter is limited to tests conducted on blood. Similarly, rapid/point of care tests are not discussed.

2.1 Standard Testing for HIV in British Columbia

The current screening test for HIV in British Columbia is an enzyme immunoassay (EIA) antibody test. EIA kits consist of HIV antigens that are bound to beads. When a serum specimen that contains HIV is added to the antigen mixture, the antibodies in the serum bind to the antigens. An enzyme linked to an antihuman antibody is added which then binds to the antibody/antigen complex. The enzyme acts on a substrate which results in colourmetric change on the emission of light in proportion to the amount of bound HIV antibody in the host. This reaction is an indication that HIV antibodies exist in the sample and an optical density or chemilummescent signal is measured. There is a direct relationship between the amount of antibody in the blood sample and the optical density (i.e. the more antibody exists, the more intense the density). There are a number of different anti-HIV EIAs available, but all of them are dependant on whether the patient's immune system has started to develop antibodies to HIV (7). The average window period from infection to antibody detectability for HIV antibody testing is

22 days (39). Antibodies can usually be detected by 22 days, but it may take up to 3 months (40).

In order to avoid risks associated with reporting a false positive, a confirmation test is required (40). In British Columbia, when HIV blood tests are EIA reactive or they yield an equivocal result, the specimen undergoes Western Blot (WB) testing (11). The Western Blot test allows specific detection of antibody to an array of viral proteins which have been attached to a strip of plastic or nitrocellulose. Based on the pattern of antibody binding to specific HIV antigens, infection is confirmed (40).

The HIV p24 antigen test is an antigen test that detects the presence of this protein in a person's blood (79% sensitive and 99% specific) (41). This test is rarely used in current practice because Nucleic Acid detection of HIV RNA (described below) has been shown to be more sensitive in detecting early HIV infection. These tests are designed to detect unbound HIV p24 antigen and do not reliably detect p24 antigen that has been complexed to antibody. In fact, p24 can usually only be detected for approximately six days in acutely infected individuals. Thus, this test may be able to detect early HIV infection at approximately three to seven days prior to the development of HIV antibodies, but once antibodies are developed the sensitivity of the test becomes poor.

2.2 Non-Standard HIV Tests

2.2.1 NAAT Testing

Nucleic acid amplification testing (NAAT) is a molecular test that detects HIV RNA (42). As mentioned in Chapter 1, HIV RNA replication is rapid and begins at the time of entry of the virus into the host. The benefit of this test is that HIV RNA detection is possible prior to

antibody development. This test is currently licensed for monitoring HIV response to therapy. It has been adopted for the detection of preseroconversion in HIV infection in developed countries. Due to its high sensitivity and specificity, NAAT testing has a short window period (1-2 weeks).

2.2.2 Avidity Testing

Avidity testing takes advantage of the fact that antibodies to HIV have less affinity for binding to HIV antigen in the first few months of infection (43). The avidity refers to the strength of binding between the antigen and antibody (44).

During avidity testing, a chaotropic agent, such as guanidine, is added to the assay which disrupts weaker antibody/antigen complexes (24). When guanidine is added to HIV positive serum, the weakly bound complexes are disrupted leaving the strongly bound complexes intact. The complexes are then detected as in the standard EIA test. Signals are assigned to the result according to the optical density or chemiluminescence signal detected. The test is also repeated using phosphate buffered saline which acts as a reference dilution. An avidity index (AI) is then calculated using a ratio of the optical density readings from the test dilution to the optical density of the reference dilution ([S/CO test dilution]/[S/CO reference dilution]) (45). The resulting value produces a continuous value which is then categorized into a dichotomous variable, using an AI of $\leq$8 as a cut-off value, to differentiate between RSC and established infections. The RSC translates into an infection that is, on average, 142 days (95% CI, 101 to 183 days) and an established infection is, on average, more that 142 days (45).

The avidity test is a desirable test for detecting RSC as it has greater reproducibility and better tests characteristics that other methods of detecting RDC (4% misclassification error with

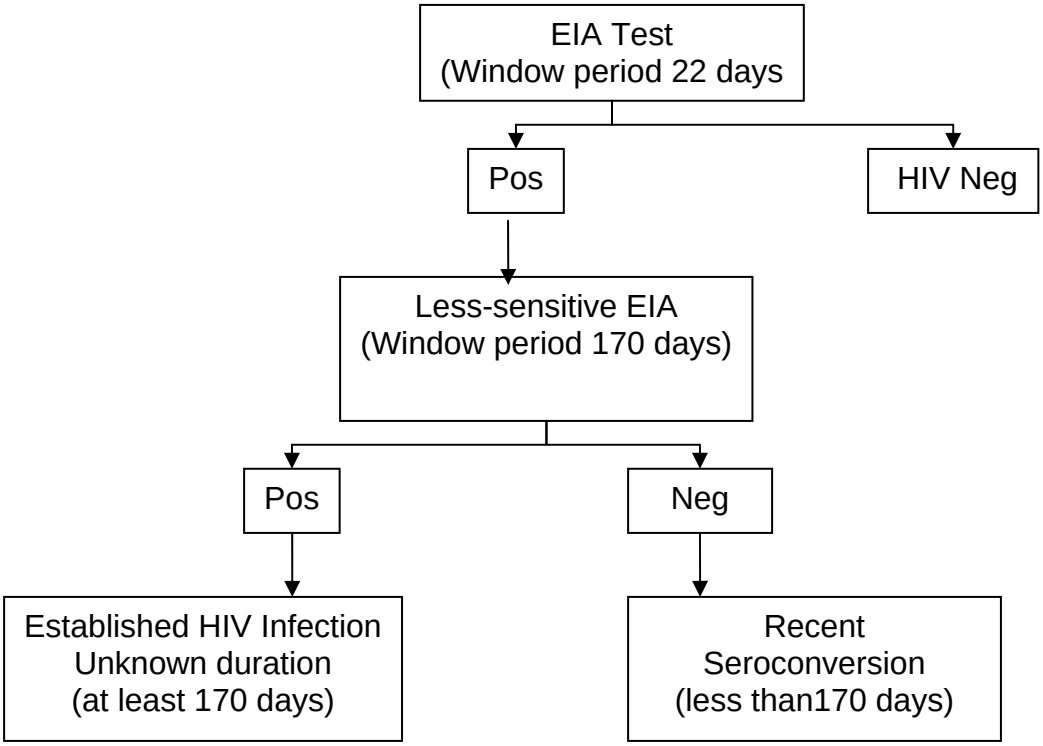
the avidity test compared to 10% with the detuned test described below (46).[46] However this method is relatively new and has only been validated using the detuned less-sensitive test as the gold standard (47). Therefore, we chose to use this so-called gold standard for our study.

2.2.3 Detuned (Sensitive/Less Sensitive) Antibody Testing to Diagnose a RSC

The detuned EIA test has been well described (15, 48-51) and is based on the fact that antibody levels are low in the early months of HIV illness and more concentrated with established illness (19). The test is conducted on serum known to be infected with HIV and employs an EIA assay that is modified by increasing the dilution of a test sample from 1:400 to 1:20,000 thus rendering it less sensitive in detecting HIV antibodies (49). Optical density readings are conducted on the diluted sample providing a continuous variable. Standard optical densities (SOD) have been calculated by the developers of the detuned test by calculating the median optical densities in test samples ÷optical densities of calibration samples.

Using a bioMerieux Vironistika assay, a SOD of less than 1 returns a "negative" result and indicates a RSC occurring within 170 days of infection (95% CI: 145 to 200) (50). Similarly, earlier detuned assays, such as the 3A11-Less Sensitive (3A11-LS) assays used a SOD of 0.75 which indicated a RSC occurring within 129 days (95% CI: 109–149 days) (49). With all versions of the detuned test, the reported result is dichotomous variable (RSC or established infections) useful for surveillance and evaluation purposes. Figure 2.1 illustrates an algorithm associated with this testing method.

Figure 2.1 Algorithm for Determining RSC Using Sensitive/Less-Sensitive Testing Methodology (15)



Detuned testing has been criticized for its relatively low sensitivity (92%) and specificity (89.5%), resulting in misclassification error. These misclassification errors mainly occur in non-B subtypes of HIV (2-8 %) (8,37,52) and in persons receiving highly active antiretroviral therapy (HAART) (8%) (38) and/or persons with AIDS (2%) (49). For example, using the 3A11-LS assay, negative detuned result indicates a recent infection $\leq$129 days for B-type HIV strains, whereas in non-B subtypes, a negative detuned result indicates an infection of 270 days to 2 years (53). In this case, an infection of over a year would likely be falsely classified as "recent". The bioMerieux Vironistika assay used in this study has the same propensity for misclassification error. However, it is assumed that misclassification errors will occur equally in each study group and therefore will not produce biased results. Table 2.1 displays the degree of misclassification errors with both the detuned test and the avidity test (46,54).

Table 2.1 Test Characteristics of the Detuned and Avidity Tests

	Detuned	Avidity
Ability to correctly identify recent HIV infection	92% (8% misclassified)	92% (8% misclassified)
Ability to correctly identify established infections	89.5% (10.5% misclassified)	98.3% (1.7% misclassified)
Ability to correctly identify established infections in cases with AIDS	87.5% (12.5% misclassified)	91.7% (8.3% misclassified)
Overall misclassification	10.4%	4.3%

CHAPTER 3: METHODS

3.1 Study Design

This study used a cross-sectional observational design. Patient data was obtained from a BCCDC reporting database and the specimens tested were from a stored repository.

3.2 Study Population

Data from 1400 new cases of HIV prior to 1 May 2003 and 1400 new cases after 1 May 2003 were extracted from the HIV surveillance database. There were no exclusion criteria for the study however only cases with serum available for detuned testing could be analyzed for the primary study objective. Cases were divided into two study groups:

Group 1: cases who became newly positive prior to 1 May 2003 (pre- reporting group)

Group 2: cases who became newly positive after 1 May 2003 (post-reporting group)

3.3 Sample Size

The proportion of RSC in Group 1 was estimated at 18% based on a published study from North Carolina (15). We hypothesized that the proportion of RSC in Group 2 will be 23% (5% difference in proportions). Based on these estimates, 1035 subjects per group are needed to have an 80% chance of rejecting the hypothesis of <5% difference at the 0.05 level. Actual calculation of sample size for comparison of 2 proportions (two-sided) was calculated per the University of London's Sample Size calculation web site (55) as follows:

$n = [Z\alpha + Z\beta]^2 * [(p1*(1-p1)) + (p2 * (1-p2))]/[p2-p1]^2$

where n = the sample size required in each group

p1 = proportion in group 1 = 0.18

p2 = proportion in group 2 = .23

p2 - p1 = size of difference of clinical importance = .05

$Z\alpha = 1.96$ where $\alpha = 0.05$

$Z\beta = 0.84$ where $\beta = 0.2$

$n = [1.96 + 0.84]^2 * [(0.18 * 0.82)) + (0.23 * 0.77))]/[0.23-0.18]^2$

$n = (7.84) * [0.15 + 0.18] / 0.05^2$

$n = (7.84)(0.33)/0.0025$

n = 1035 per group

The study population included 1035 cases per arm. An additional 18% was added to the study sample to allow for cases that had inadequate amounts of serum therefore the final sample was 1400 cases per arm.

3.4 Subject Identification

Subjects with confirmed newly positive HIV tests were identified by the HIV data analysts at STI/HIV Prevention and Control through an access query. This included serologically confirmed cases of HIV only.

Classification into study groups was done according to the date the lab received the sample for HIV testing. If this date was on or before 1 May 2003 participants were stratified into Group 1. If this date was after 1 May 2003 participants were stratified into Group 2. Date of receipt of sample by the lab was used because it is the date that is available in 100% of the cases compared to the date the lab sample was taken from the client which is not always provided by

the health care provider. Since serum is generally sent to the Provincial lab within a day of collection, this would not have created a bias.

3.5 Identification of Lab Samples for Detuned Testing

All blood received by the HIV testing laboratory is given a unique specimen number. This specimen number is entered into the BC HIV Surveillance System (HIVSS) database consisting of all data collected for case reporting. The date of the specimen and the test result is also entered into the HIVSS database. Specimen numbers for all study were extracted from the HIVSS database and provided to the lab to identify matched serum samples for detuned testing.

3.6 Classification of 'Recent' Seroconversion

In BC, once client serum is fully tested for HIV (including confirmatory testing), the remaining serum is banked in the laboratory for future purposes. For the purposes of this study, all WB reactive banked serum or plasma was re-tested, by a Health Canada laboratory, using a detuned test (described in Chapter 2). Serum that was WB reactive but was detuned "negative" (i.e. SOD<1) was classified as a RSC (i.e. infection occurring less than 170 days [95% CI, 145–200 days] of the date they were tested) (50).[50] Serum that was WB reactive and detuned positive (SOD >1) was classified as an established infection of more than 170 days. This provided a dichotomous outcome variable.

Previous researchers have reported that 2-10% of people with AIDS may falsely identify as recently infected due to their declining antibody levels, according to detuned testing (42-56). Prior to any analysis, cases that were found to be in the AIDS reporting database were classified as established HIV illness rather than RSC irregardless of their detuned result.

3.7 Collection of Clinical Data

Variables selected for use with this analysis were generated after comprehensive review of the literature pertaining to early or recent HIV infections. Factors that have been demonstrated to impact on HIV test performance, HIV testing patterns, and socio-demographic characteristics of HIV positive individuals, as well as associated risk behaviours, were considered. As part of HIV reporting Clinical information pertaining to all new cases of HIV is gathered by public health professionals or physicians and submitted to the BC HIV surveillance nurses at the BCCDC on a case report form (CRF). The data is then entered into the HIVSS database. For the purposes of this study, results from the detuned test were linked to the HIVSS database by the STD/AIDs Control Surveillance Analyst. Once the linkage was complete, all personal identifiers were removed from the dataset before it was handed over to the researchers.

The following variables were extracted from the HIVSS database:

1. Clients name, initials, and date of birth (for linking purposes)
2. Gender: male, female or transgendered
3. Ethnicity (as documented on the CRF): self-described by individuals. First Nations, Metis and Inuit have been collapsed into one category called Aboriginal. Asian individuals included anyone who was from South Asian (India, Pakistan, Bangladesh etc), as well as South East Asia (Thailand, Phillipines, Indonesia etc) and Asia (China Japan, Korea, etc).
4. Date of first positive HIV test
5. Date of most recent negative HIV test
6. Health Service delivery area where the HIV test was done.
7. Risk factors including:

a. partner of HIV+ person
b. sexual orientation
c. sex trade worker
d. patron of sex trade worker
e. injection drug user
f. recipient of blood or blood products
g. occupation exposure
h. and endemic country

8. Primary Reason for Testing for HIV:
    a. Symptomatic
    b. Evidence of seroconversion
    c. Client requested a test
    d. Sexual contact of an HIV infected person
    e. Partner is HIV positive
    f. Immigration test
    g. Comes from a country where HIV is endemic
    h. Prenatal test
    i. Has other STI
    j. Tested as part of research study
    k. Reason is unknown
9. Health at time of test: classification of HIV disease staging as documented by the health care provider who completed the HIV Reporting case report form. This includes:
    a. Acute seroconversion

b. Asymptomatic

c. Late HIV illness

d. AIDS defining illness

10. Diagnosis and date of diagnosis of AIDS (if applicable) as recorded in the AIDS Reporting Database at BCCDC.
11. CD4 count and viral load counts at time of testing as reported on the case report form

3.8 Statistical Methods

All statistical analysis was completed using SPSS 14.0 for Windows.

3.8.1 Description of Overall Population and Two Study Groups

Frequency analysis was conducted on categorical demographic information (gender, ethnicity, age) and risk factor data. Frequencies of cases presenting with AIDS at the time of first HIV test, subtype of HIV strain, and primary drug resistance was also reported. Mean and standard deviation (SD) were used to describe normally distributed continuous variables (age, time since last negative test, viral load). Medians, intra-quartile range, and minimum maximum values were calculated to describe skewed continuous variables. Newly positive cases which did not have a sufficient quantity of serum available for detuned assay were marked as such. These cases were excluded in the analysis for the primary and secondary objectives.

Year of diagnosis was broken down into quarters. The rate of reported new cases per 100,000 in BC per quarter over the study period was graphed to study a seasonal effect, if present. Frequency of HIV tests during the same time periods was also calculated and graphed.

Bivariate analysis was conducted on pre-reporting and post-reporting groups using Chi-square tests (categorical data), t-tests (normally distributed continuous data), and Mann -Whitney

U tests (skewed continuous data) to detect heterogeneity, if any, between groups in terms of demographic and sexual risk factors. Differences in characteristics between groups were taken into consideration when interpreting the results of the study.

A two-side z-test, as described by Pagano and Gauvreau (57), was conducted to detect a difference, if any, in the proportion of RSC between groups. This is a test of independent proportions. The assumption for this test is that the test statistic has a normal distribution. The distribution considered normal if each of the quantities $n_1 p^$, $n_1(1-p^)$, $n_2 p^$, and $n_2(1-p^)$ are greater than five; or more simply put, if the number of RSC cases and late seroconverters (LSC) in both study groups are all greater than 5. The null hypothesis is that the proportion of RSC before HIV Reporting is equal to the proportion after HIV Reporting (H_0: $p_1 = p_2$). The test statistic was calculated as follows:

$$z = \frac{(p^_1 - p^_2) - (p_1 - p_2)}{\sqrt{p^(1 - p^)\left[(1/n_1) + (1/n_2)\right]}}$$

The a priori decision was that if the p-values associated with the calculated test statistic is less than 0.05, then the null hypothesis would be rejected. Proportions of RSC were calculated with 95% confidence intervals. The prevalence of recent infections was assumed to be similar among HIV-positive individuals who were not detuned tested (19). A sensitivity analysis was conducted by repeating the z-test with a subset of cases that were all B subtype to determine if the non-B subtype samples (most likely cause of misclassification from detuned testing in this population) may have introduced spurious results.

This analysis was conducted again using the proportion of cases with a diagnosis of AIDS at the time of their first positive HIV test before and after HIV Reporting. AIDS at the

time for first positive was determined by matching the HIVSS database with the AIDS reporting database. Reported cases of AIDS that were within 3 months of the date of a new HIV diagnosis were considered as having AIDS at the time of HIV diagnosis. The null hypothesis was that the proportion of newly positive AIDS cases after HIV Reporting is equal to the proportion before HIV Reporting.

A Kappa test (58) was conducted on the staging classification documented in the CRF and the staging classification based on the detuned results. For this analysis, staging classification was collapsed into a dichotomous variable as seroconversion and non-seroconversion variables (non-seroconversion = asypmptomatic, late stage HIV and AIDS). Interpretation of the results is consistent with those described by Landis and Koch (59). The statistic was interpreted as follows: < 0.00 = poor agreement; 0.00–0.20 = slight agreement; 0.21-0.40 = fair agreement; 0.41-0.60 = moderate agreement; 0.61-0.8 = substantial agreement; and 0.81-1.00 = almost perfect agreement.

Logistic regression analysis was used to model the association between independent variables and RSC status at the time of their first positive HIV test, with the odds ratio being a measure of association. The assumptions for this test are that the outcome variable is binary and continuous variables must conform to linear gradient (60). In addition, multicollinearity must not be present (61).

The variable RSC status was used as the dependant variable and demographic/risk variables were used as potential predictor variables. Variables, such as age, gender, ethnicity, and sexual orientation have been shown previously to be associated with RSC. (22-23,62). These variables were entered into the model based on this previous research. Bivariate analysis was conducted using a chi-square test to determine which of the other variables were

significantly associated with being a RSC at the time of testing. Crude odds ratios were calculated for the bivariate associations. Variables that were significantly associated in the bivariate analysis, and those commonly known to act as confounders in regression models (i.e. gender) were entered into the logistic regression model in a forward step-wise manner. Independent variables with a large number of missing values were excluded from the analysis as this would reduce the power of the model. Variables were entered manually, rather than through an automatic computerized mechanism.

Reference groups were decided upon a priori (presented in results section). Variables reaching a significance value of 0.1 were included in the model and considered explanatory variables associated with being a RSC. The adjusted odds ratio was a measure of association. A Likelihood Ratio (LR) test was used to test the significance of the model. The number of variables entered into the model was limited to the number of events (RSC = an event) divided by 10 (63). The results of the LR test were considered after each variable was added to the model.

3.8.2 Confounding

Confounding was considered to be present when the coefficient of the previous variables changed dramatically when another variable is introduced into the model. In addition, confounding was detected by comparing the crude odds ratio to the adjusted odds ratio. If these two differ by 10% or more, then it was determined that confounding is present (61). If less than 10%, then it was considered that there was not an important amount of confounding.

3.8.3 Collinearity

Chi-square tests were also conducted between independent variables when collinearity was suspected. If two independent variables were strongly associated, only one was chosen for entry into the model. This was done to avoid imprecise estimates of the coefficients and inaccurate variance associated with the coefficients (60). SPSS does not test for collinearity, so detection of this was done manually while the model was created.

3.8.4 Interaction

Interaction occurs when the relationship between a independent variable and the dependent variable depends on the level of a third variable (64). In essence, interaction can cause an overestimation or underestimation of the effect size. The presence of interaction terms was examined after each variable was entered into the model. When the addition of a variable caused a previously significant coefficient to become non-significant then an interaction was thought to have occurred. Interaction terms were entered into the model and the significance of the interaction was measured and reported (60).

3.8.5 Sensitivity Analysis

A proportion of HIV positive study samples were not found in the serum archives or they lacked sufficient quantity to perform the detuned test. Demographic and risk factors for these samples were compared to the demographic and risk factors for cases for which serum was available. This was done to determine if the missing samples introduced a systematic bias. The comparison was done by conducting chi-square tests on categorical data, t-tests on normally distributed continuous data, and Mann-Whitney U tests on skewed continuous data. The

assumptions for the Mann-Whitney U test are that the two samples must be independent, and that the observations to be ordinal or continuous measurements.

The problem of "time" as an independent variable possibly affecting incidence of HIV was considered by considering a time-series analysis. The main assumption of times-series analyses is that autocorrelation must be present. This means that incidence in one time period is dependent on the incidence in the time period(s) preceding it (65-70). Tests for autocorrelation were conducted with both proportions and numbers of RSC per year (with and without log transformation and differencing) over time to determine if a time series analysis could be conducted. These tests revealed there was no autocorrelation over time violating the basic assumption of time series analysis and therefore a time series analysis was not performed.

3.9 Inherent Limitations

This study was designed to examine the association between HIV Reporting and being recently infected at the time of HIV diagnosis. It was not intended to prove a casual relationship between these two factors.

The limitations around temporality normally seen with cross-sectional studies were not present because we had the actual dates for both the onset of HIV Reporting and the date of newly positive HIV test. The other limitation normally seen in cross-sectional designs includes selection bias. Selection bias may exist due to the exclusion of cases which did not undergo detuned testing. As mentioned above, a sensitivity analysis was conducted to determine if the characteristics of the people for whom serum was available are significantly different than those for whom serum was not available using a chi-square test for independence (71). In addition,

there were limitations to using existing databases due to missing data which may be missing systematically, creating a bias of unknown origin.

Finally, the potential for misclassification error due to the test characteristics of the detuned test may create spurious results. Erroneous estimates in the number of incident cases is assumed to be equal between study groups and therefore should not affect the primary objective. However, it may affect all three secondary objectives. Therefore sensitivity analyses were completed.

CHAPTER 4: RESULTS

4.1 HIV Positive Cases Before and After HIV Reporting

Between 1 Jan 2000 and 23 August 2006, 982,222 people were tested for HIV and among these, there were 2728 reported cases of HIV in British Columbia; 1368 (50.1%) pre-HIV reporting and 1360 (49.9%) post-HIV reporting. Figures 4.1 and 4.2 display the trends in testing and new cases of HIV in BC over time.

Generally speaking, there was an upward trend in HIV testing between 2000 and 2005 while the number of new cases of HIV remained constant. The demographic and risk factor breakdown of HIV positives before and after Reporting can be found in Table 4.1.

Results of the bivariate analysis shown in Table 4.1 reveal that, although there is no statistical difference in gender and ethnicity breakdown between study groups, the pre-reporting group is significantly younger than the post-reporting group (38 yrs. compared to 39 yrs. respectively; p=.001) and there are significant differences in risk behaviours between groups. The difference in age is statistically significant but not clinically significant. Specifically, there is a higher proportion of MSM and sex workers in the post-reporting group with a smaller proportion of IDUs in the post-reporting group. In addition, there is a significantly larger proportion of sex workers which were also IDUs in the post-reporting group (6.7% post-reporting compared to 4.2% pre-reporting; p=<.001).

4.2 Serum Available for Detuned Testing

Among all individuals with confirmed HIV using Western Blot testing, material was insufficient for detuned testing in 1147 (42%) samples (257 [18.8%] Pre-Reporting and 890 [65.4%] Post-Reporting). Table 4.2 reports the results of bivariate comparison of demographic and risk

Figure 4.1 Trend of HIV Testing Over Time

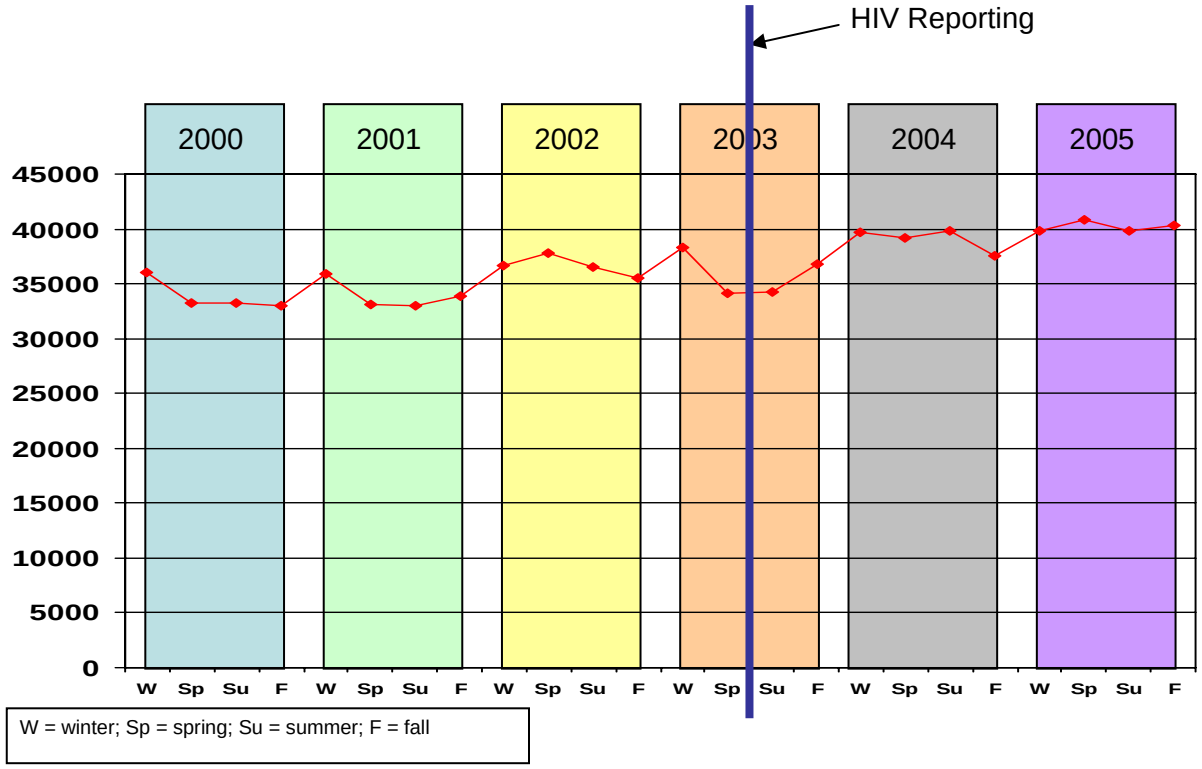


Figure 4.2 Trend of Newly Positive Cases of HIV Over Time

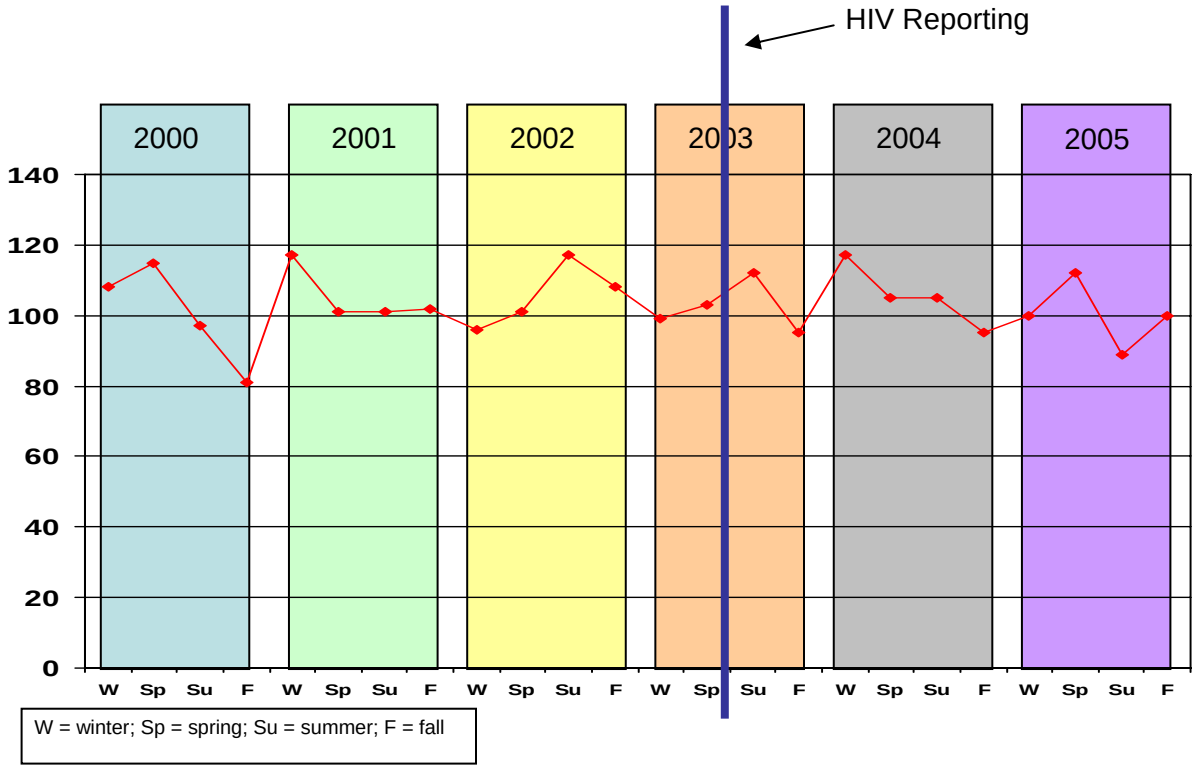


Table 4.1 Demographic and Risk Factor Breakdown of HIV Positives Before and After Reporting

	Pre Reporting Group n = 1368 (%; 95% CI))	**Post Reporting Group n = 1360 (%; 95% CI))**	**All n = 2728 (%; 95% CI))**	**Significance between groups (2-sided)**
Gender[ж] (%; Binomial CI)				
Male	1086 (79.8%; CI: 77.6, 81.9)	1063 (78.3; CI:76.1, 80.5)	2149 (78.8%; CI:77.5, 80.6)	p = 0.37 (Pearson χ^2 test)
Female	275 (20.2%; CI: 18.1, 22.4)	294 (21.7%; CI:19.5, 24.0)	569 (20.9%; CI:19.4, 22.5)	
Age				
mean; (SD; range)	38.2 (10.7; 5-79)	39.5 (11.1; 2-82)	38.9 yrs (10.9; 2-82)	**p = .001** (independent t-test)
Race/Ethnicity[⌘] (%; Binomial CI)				
Aboriginal	211 (16.3%; CI:14.3, 18.4)	195 (15.6%; CI: 13.8, 18)	406 (16.1%; CI: 14.7, 17.6)	p = 0.63 (Mann-Whitney U)
Asian (any)	103 (7.9%; CI:6.5, 9.6)	100 (7.4%; CI: 6.7, 9.8)	203 (8%; CI: 7, 9.2)	
Black	64 (4.9%; CI:3.8, 6.3)	56 (4.5%; CI: 3.5, 5.9)	120 (4.7%; CI: 4, 5.7)	
Caucasian	872 (67.2%; CI:64.6, 69.78)	830 (67.4; CI: 64.7, 70)	1702 (67.3%; CI: 65.5, 69.2)	
Hispanic	43 (3.3%; CI:2.4, 4.4)	44 (3.5%; CI: 2.5, 4.7)	87 (3.4%; CI: 2.8, 4.2)	
Other/Mixed	4 (0.3%; CI:0.08, 0.8)	6 (0.5%; CI: 0.2, 1.1)	10 (0.4%; CI: 0.2, 0.7)	

Table 4.1 Continued

	Pre Reporting Group n = 1368 (%; 95% CI))	**Post Reporting Group n = 1360 (%; 95% CI))**	**All n = 2728 (%; 95% CI))**	**Significance between groups (2-sided)**
Risks (not mutally exclusive)Ψ (%; Binomial CI)				
MSM	489 (35.8%; CI: 33.2, 38.4)			**p = 0.012**
MSM and IDU	42 (3.1%; CI: 2.2, 4.1)	550 (40.4%; CI:37.8, 43.1)	1039 (38.1%; CI:36.3, 40)	p = 0.64
IDU	501 (36.6%; 34.1, 39.2)	39 (2.9%; CI:2.1, 3.9)	81 (3.0%; CI:2.4, 3.7)	**p = 0.001**
Sex Worker	69 (5.0%; CI: 4.0, 6.3)	413 (30.4%; CI: 27.9, 32.9)	914 (33.5%; CI: 31.7, 35.3)	**p = 0.003**
Sex Worker and IDU	58 (4.2%; CI: 3.2, 5.5)	107 (7.9%; CI: 6.5, 9.4)	176 (6.5%; CI: 5.6, 7.4)	**p = <.001**
		91 (6.7%; CI: 5.4, 8.2)	149 (5.5%; CI: 4.6, 6.4)	
HIV Sub-type**				
B	495 (96.5%; CI: 9.5, 9.8)	304 (91.3%; CI: 87.7, 94.1)	799 (94.4%; CI: 92.7, 95.9)	**p = 0 .002**
Non-B	18 (3.5%; CI: 2.0, 5.5)	29 (8.7%; CI: 5.9, 12.3)	47 (5.6%; CI: 4.1, 7.3)	
Primary HIV Drug Resistance***				
Yes	40 (8.0%; CI: 5.8, 10.7)	24 (7.3%; CI: 4.7, 10.6)	64 (7.7%; CI: 6.0, 9.7)	p = 0.89
No	461 (92%; CI: 89.3, 94.2)	307 (92.6%; CI: 89.4, 95.3)	768 (92.3%; CI: 90.9, 94.0)	
AIDS at time of first pos HIV test				
Yes	91 (6.7%; CI: 5.4, 8.1)	104 (7.6%; CI: 6.3, 9.2)	195 (7.1%; CI: 6.2, 8.2)	p = 0.61
No	1277 (93.3%; CI: 91.9, 94.6)	1256 (92.4%; CI: 90.8, 93.7)	2533 (92.9%; CI: 91.8, 93.8)	

[Ж]excludes 9 persons for whom gender was unknown and 1 transgendered person

[⌘]self-reported ethnicity; excludes 200 persons from whom ethnicity was unknown

♌ Single risk factor, based on higherarchy of exposures

** excludes 855 for whom HIV Sub-type is unknown

*** excludes 867 for whom Primary HIV Drug resistance is known

Ψ self-reported risk; Not mutually exclusive groups; χ^2 done for each category

Table 4.2 Results of Bivariate Comparison of Cases That Were Detune Tested and Cases That Were Not Detuned Tested

	Pre Reporting n = 1368			Post Reporting n = 1360			All n = 2728		
	Detune n= 1111	**No Detune n= 257**	**P**	**Detune n= 470**	**No Detune n= 890**	**P**	**Detune n=1581**	**No Detune n= 1147**	**P**
Gender									
Male	887 (79.8%)	199 (77.4%)	0.44	361 (76.8%)	702 (78.9%)	0.32	1248 (79.1%)	901 (78.9%)	0.68
Female	219 (19.7%)	56 (21.8%)		109 (23.2%)	185 (20.8%)		328 (20.8%)	241 (21.1%)	
Age (yrs)									
Mean	38.29	37.77	0.49	39.27	39.67	0.53	39.25	38.58	0.12
Race/Ethnicity									
Aboriginal	160 (14.4%)	51 (19.8%)	0.08	68 (14.5%)	127 (14.3%)	0.82	228 (14.5%)	178 (15.6%)	
Asian (any)	81 (7.3%)	22 (8.6%)		32 (6.8%)	68 (7.6%)		113 (7.2%)	90 (7.9%)	
Black	57 (5.1%)	7 (2.7%)		22 (4.7%)	34 (3.8%)		79 (5.0%)	41 (3.6%)	**0.04**
Caucasian	713 (64.2%)	159 (61.9%)		307 (65.3%)	523 (58.8%)		1020 (64.7%)	682 (59.7%)	
Hispanic	39 (3.5%)	4 (1.6%)		13 (2.8%)	31 (3.5%)		52 (3.3%)	35 (3.1%)	
Other/Mixed	3 (0.3%)	1 (0.4%)		2 (0.4%)	4 (0.4%)		5 (0.3%)	5 (0.4%)	
Risks (Primary Risk) ♂♀ (%;)									
MSM	364 (32.8%)	85 (32.3%)		169 (40.0%)	348 (39.1%)		533 (33.8%)	433 (37.9%)	
MSM and IDU	38 (3.4%)	5 (1.9%)	**<.001**	18 (3.8%)	26 (2.9%)	0.29	56 (3.6%)	31 (2.7%)	**<0.001**
IDU	339 (30.5%)	68 (26.5%)		116 (24.7%)	175 (19.7%)		455 (28.9%)	243 (21.3%)	
Sex Worker	1 (0.09%)	1 (0.39%)		2 (0.43%)	3 (0.34%)		3 (0.19%)	4 (0.35%)	
Sex Worker and IDU	29 (2.6%)	22 (8.6%)		29 (6.2%)	50 (5.6%)		58 (3.7%)	72 (6.3%)	
Other and Unknown	340 (30.6%)	78 (30.4%)		136 (29.0%)	285 (32.4%)		476 (30.2%)	366 (32.0%)	

Table 4.2 Continued

	Pre Reporting n = 1368			Post Reporting n = 1360			All n = 2728		
	Detune n= 1111	**No Detune n= 257**	**P**	**Detune n= 470**	**No Detune n= 890**	**P**	**Detune n=1581**	**No Detune n= 1147**	**P**
Risks (not mutally exclusive)Ψ (%)									
MSM			0.48			0.64			
MSM and IDU	402 (36.2%)	87 (33.9%)	0.10	186 (39.6%)	364 (40.9%)	0.52	588 (37.3%)	451 (39.5%)	0.26
IDU	38 (3.4%)	4 (1.6%)	0.90	17 (3.6%)	22 (2.5%)	**0.03**	55 (3.5%)	26 (2.3%)	**0.03**
Sex Worker	406 (36.5%)	95 (37.0%)	**<.001**	160 (34.0%)	253 (28.4%)	0.40	566 (35.9%)	348 (30.5%)	**0.003**
Sex Worker and IDU	42 (3.8%)	27 (10.5%)	**<.001**	41 (8.7%)	66 (7.4%)	0.95	83 (5.3%)	93 (8.1%)	**0.003**
	35 (3.2%)	23 (8.9%)		35 (7.4%)	56 (6.3%)		70 (4.4%)	79 (6.9%)	0.9
HIV Sub-type*									
B	494 (44.5%)	1 (0.4%)	1.00	278 (59.1%)	26 (2.9%)	**.034**	772 (49.0%)	27 (2.4%)	**.001**
Non-B	18 (1.6%)	0		23 (4.9%)	6 (0.7%)		41 (2.6%)	6 (0.5%)	
Primary HIV Drug Resistance*									
Yes	40 (3.6%)	0	0.77	23 (4.9%)	1 (0.1%)	0.49	63 (4.0%)	1 (0.1%)	0.31
No	460 (41.4%)	1 (0.4%)		276 (58.7%)	31 (3.5%)		736 (46.7%)	32 (2.8%)	
AIDS at time of first pos HIV test**									
Yes	74 (6.7%)	17 (6.6%)	0.98	35 (7.4%)	69 (7.8%)	0.84	109 (6.9%)	86 (7.5%)	0.55
No	1037 (93.3%)	240 (93.4%)		435 (92.6%)	821 (92.2%)		1472 (93.3)	1061 (92.9%)	

* excludes cases from 2002 and 2003 as no sub-type and drug resistance info available

** includes cases where AIDS was diagnosed within 3 months of first HIV positive test

*** self-reported risk; Not mutually exclusive groups; χ^2 done for each category

ℌ Single risk factor likely contributing to greatest exposure to HIV

Ψ self-reported risk; Not mutually exclusive groups; χ^2 done for each category

behaviour characteristics between cases that were detune tested and cases that were not detuned tested.

Results of the bivariate analysis above reveal that there is no statistical difference in age, gender, primary drug resistance, and AIDS at time of first positive HIV test between the cases that were detuned tested and those not detuned tested. However, the subset of detuned tested cases represent a greater proportion of IDU and MSM/IDU and a lower proportion of sex workers (IDU and non-IDU) than the cases that did not undergo detuned testing. When broken down according to study group this bias appears equally distributed between groups, although, there is a possibility of a Type II error.

Overall, 846 (31%) underwent HIV subtyping [pre-reporting group: 513 (37.5%); post-reporting group: 333 (24.5%)]. There appears to be a lower proportion of non-B subtype in the subset that was detuned tested (detuned: 5% non-B; non-detuned: 18% non-B) which may result in a lower misclassification rate than if all the cases had been detuned tested. The post-reporting group has a higher proportion of non-B samples (4.9%) than the pre-reporting (1.6%) group which may have caused a greater overestimation of RSC in the post-reporting group than the pre-reporting group. This is because non-B subtypes that are truly established are more likely to be classified as "recent" infections by the detuned testing method (53).

Among the detuned samples, 109 cases were known to have AIDS illness at the time of their first positive HIV test according to the AIDS reporting database. Twenty-nine (26.6%) were misclassified as "recent infections" according to the detuned test result. These men were re-classified as having established HIV illness in the dataset. Subtype information was available on 18 of the misclassified cases revealing only one non-B subtype (subtype C). The remaining 17 were from subtype B specimens.

4.3 Rate of Recent Infections

A total of 1581 cases were detuned tested, 1111 in the pre-reporting group, and 470 in the post-reporting group. Overall among these, 447 (28.3%; CI: 26.1, 30.6) were classified as recently infected (i.e. within 170 days of infection) 311 (28%; CI: 25.4, 30.7) were from the pre-reporting group and 136 (29%; CI: 24.9, 33.3) were from the post-reporting group.

4.4 Two-Side Test of Proportions

Proportions per study group were calculated as the number of RSC (according to detuned test results) divided by the total number of detuned tests conducted. The proportion of recent infections in the pre-reporting group was 28% (95% CI: 25.4%, 30.7%) and the proportion in the post-reporting group was 29% (95% CI: 24.9%, 33.23%). As mentioned in Chapter 3, since the number of RSC cases and LSC in both study groups are greater than 5 the data can be considered normally distributed and a parametric test is appropriate. The z-test statistic was calculated as follows:

$$z = \frac{(\hat{p}_1 - \hat{p}_2)}{\sqrt{\hat{p}(1-\hat{p})\left[(1/n_1) + (1/n_2)\right]}}$$

$$z = \frac{(0.28 - 0.29)}{\sqrt{0.2827 * 0.7173 * \left[(1/1111) + (1/470)\right]}}$$

$$z = \frac{-0.01}{\sqrt{0.2827 * 0.7173 * 0.003}}$$

$$z = \frac{-0.01}{0.2478} = -0.40356$$

The p value associated with z = -0.4356 is p = 0.703.

Figure 4.3 shows the trend in rates of RSC over time in relation to the start of HIV Reporting.

Figure 4.3 Trend of Recent HIV Cases Over Time

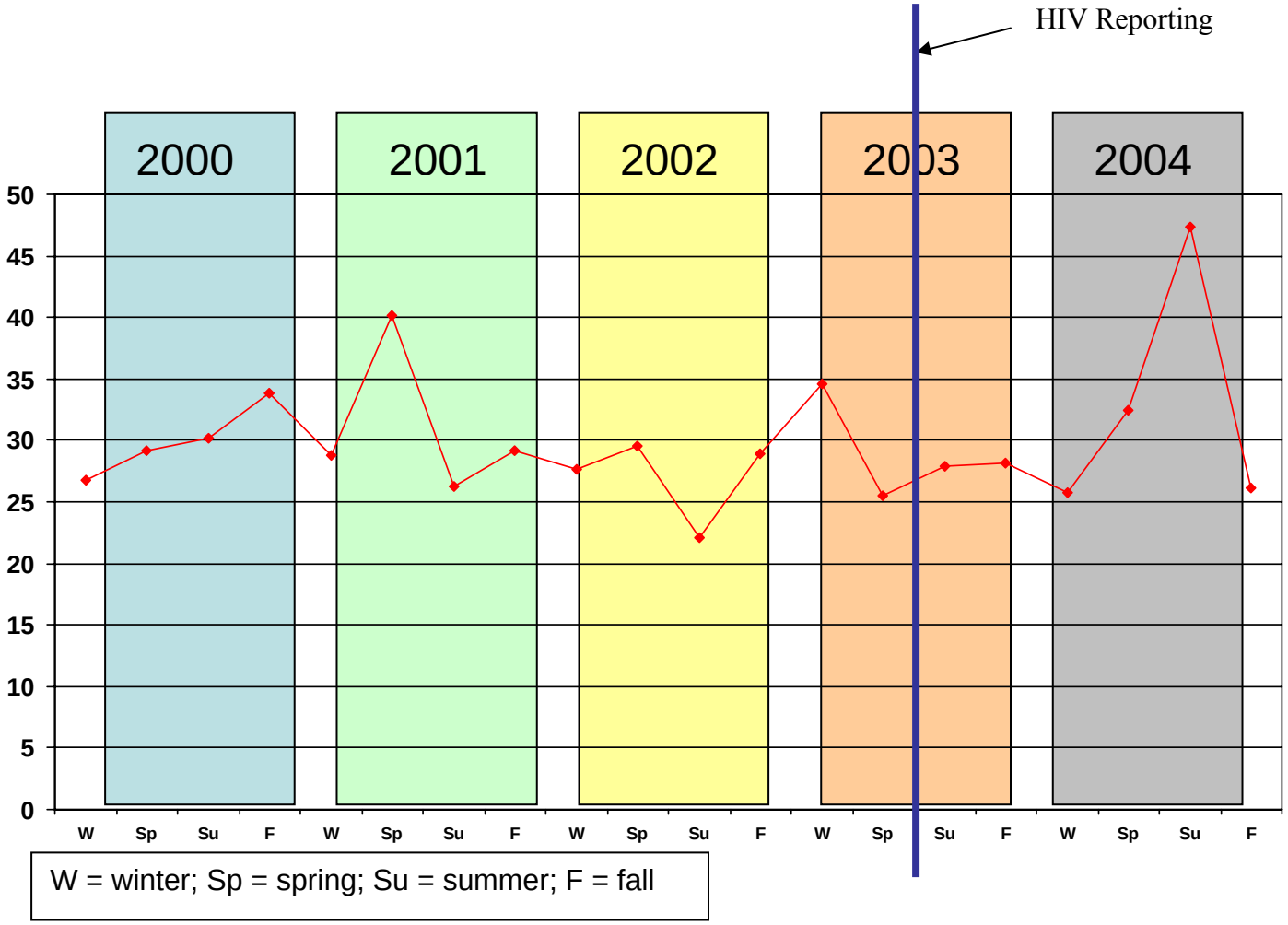


Figure 4.3 reveals that there are fluctuations in the rate of recent infections per quarter (ranging from 22% to 27%) but that there does not appear to be a systematic seasonal pattern.

Table 4.3 displays the proportion of recent infections in relation to the number of HIV tests and the overall number of HIV positive reports per quarter.

4.5 Risk Behaviour in Recent and Established Cases of HIV

Overall, 179 (30.4%) MSM tested by the detuned test were recently infected [pre-reporting: 121 (30.1%); post-reporting: 58 (31.2%) p=0.84]. Among the 83 sex trade workers tested by the detune test, 40 (48.2%) were recently infected [pre-reporting: 22 (52.4%); post-reporting: 18 (43.9%) p=0.7]. Finally, among the 566 cases that self-reported as being an injection drug user, 192 (33.9%) were recently infected [pre-reporting: 135 (33.3%); post-reporting: 57 (35.6%) p=0.62]. It should be noted that even though none of these differences were statistically different, the sample size in each of these groups may not have been large enough to sufficiently power the detection of a difference. Figure 4.4 displays the proportion of recent infections among various high risk groups over time in each study group.

4.6 Proportion of Newly Reported Cases of HIV with AIDS at Time of Diagnosis

The frequency of newly reported cases of HIV presenting with a clinical picture consistent with AIDS is displayed Figure 4.5.

Table 4.3 Breakdown, By Quarter, of HIV Seroprevalence and Proportion of Recent Infections in British Columbia Over Time.

	Persons testing for HIV **A**	New HIV+ reports **B**	Rate of pos tests per 10,000 tests **(B/A)*10,000**	Number of samples tested using detuned testing method (%) **C**	Number of recent infections **D**	Proportion (95% CI) of recent infections among samples detuned tested **D/C**
2000 (total)						
Q1	36010	108	30.0	97 (89.8%)	26	26.8 (18.3-36.7)
Q2	33306	115	34.5	95 (82.6%)	27	28.4 (19.6-38.6)
Q3	33224	97	29.2	86 (88.7%)	25	29.1 (19.8-39.9)
Q4	32948	81	24.6	74 (91.4%)	23	31.1 (20.8-42.9)
2001 (total)						
Q1	35923	117	32.6	104 (88.9%)	26	25.0 (17.0-34.5)
Q2	33055	101	30.6	87 (86.1%)	34	39.1 (28.8-50.1)
Q3	32964	101	30.6	76 (75.2%)	20	26.3 (16.9-37.7)
Q4	33863	102	30.1	89 (87.3%)	25	28.1 (19.1-38.6)
2002 (total)						
Q1	36626	96	26.2	83 (86.5%)	22	26.5 (17.4-37.3)
Q2	37808	101	26.7	71 (70.3%)	18	25.4 (15.8-37.1)
Q3	36520	117	32.0	95 (81.2%)	19	20.0 (12.5-29.5)
Q4	35537	108	30.4	97 (89.8%)	27	27.8 (19.2-37.9)
2003 (total)						
Q1	38241	99	25.9	52 (52.5%)	18	34.6 (22.0-49.1)
Q2	34146	103	30.2	47 (45.6%)	11	23.4 (12.3-38.0)
Start of HIV Reporting						
Q3	34208	112	32.7	43 (38.4%)	11	25.6 (13.5-41.2)
Q4	36744	95	25.9	78 (82.1%)	21	26.9 (17.5-38.2)

Table 4.3 Continued

	Persons testing for HIV **A**	New HIV+ reports **B**	Rate of pos tests per 10,000 tests **(B/A)*10,000**	Number of samples tested using detuned testing method (%) **C**	Number of recent infections **D**	Proportion (95% CI) of recent infections among samples detuned tested **D/C**
2004 (total)						
Q1	39650	117	29.5	66 (56.4%)	17	25.8 (15.8-38.0)
Q2	39229	105	26.8	80 (76.2%)	26	32.5 (22.5-43.9)
Q3	39853	105	26.3	93 (88.6%)	36	38.7 (38.8-49.4)
Q4	37533	95	25.3	68 (71.6%)	15	22.1 (12.9-33.8)
2005 (total)						
Q1	39826	100	25.1	No detuned testing done this year	N/A	N/A
Q2	40847	112	27.4			
Q3	39853	89	22.3			
Q4	40350	100	24.8			

Q1 = 1 Jan – 31 Mar; Q2 = 1 Apr – 30 June; Q3 = 1 Jul – 30 Sep; Q4 = 1 Oct – 31 Dec

Figure 4.4 The Proportion of Recent Infections Attributable to High Risk Groups

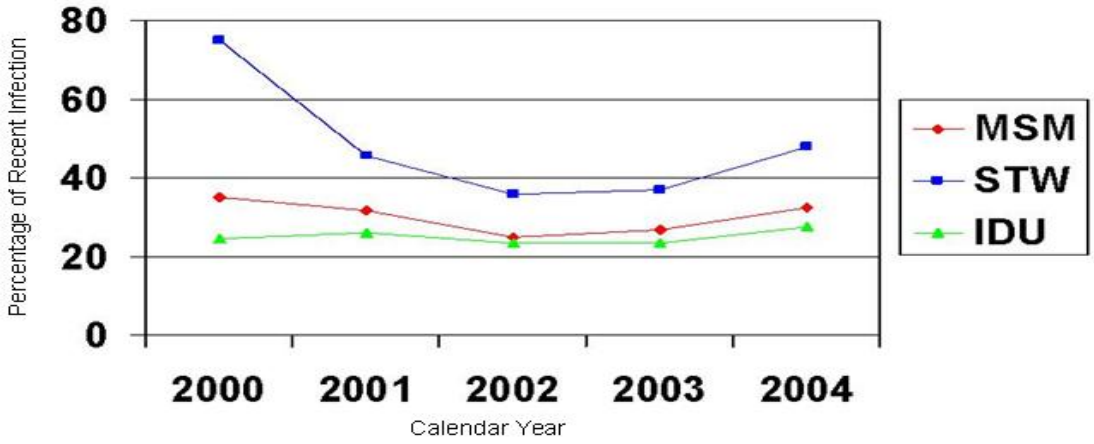
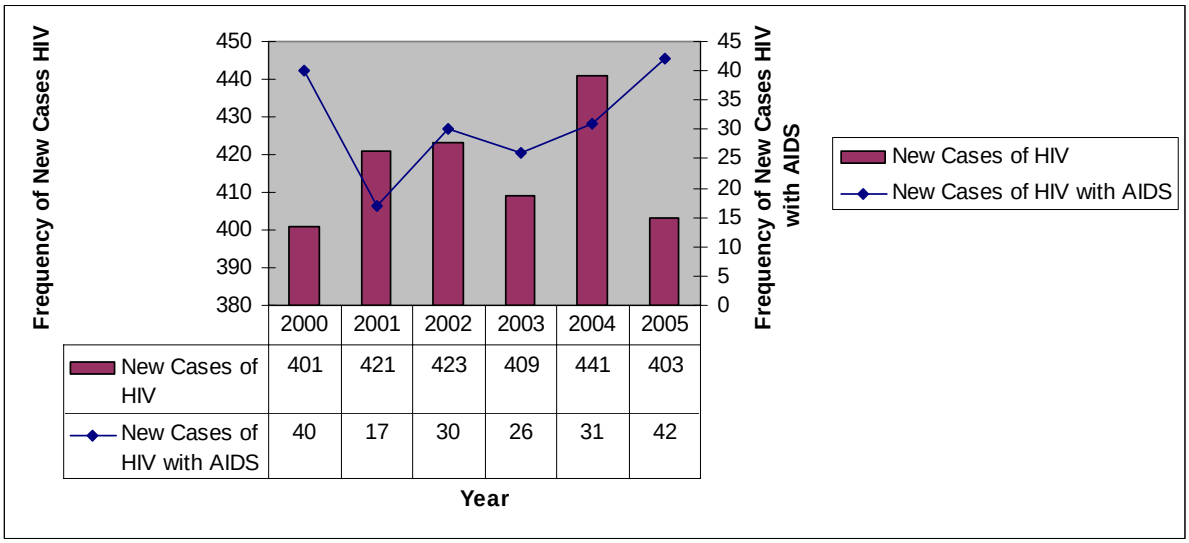


Figure 4.5 Newly Reported Cases of HIV Presenting With a Clinical Picture Consistent With AIDS



Overall, between Jan 2000 and Aug 2005, 195 (7.1%) of newly reported HIV cases had a clinical picture consistent with the diagnosis of AIDS. The number of AIDS cases in the pre-reporting group was 91 (6.7% [95% CI: 5.4%, 8.1%]) and the number of AIDS cases in the post-reporting group was 104 (7.6% (95% CI: 6.3%, 9.1%) (p=0.313). The z-test statistic was calculated as follows:

$$z = \frac{(\hat{p}_1 - \hat{p}_2)}{\sqrt{\hat{p}(1-\hat{p})\left[(1/n_1) + (1/n_2)\right]}}$$

$$z = \frac{(0.067 - 0.076)}{\sqrt{0.071 * 0.929 * \left[(1/1368) + (1/1360)\right]}}$$

$$z = \frac{-0.009}{\sqrt{0.071 * 0.929 * 0.0015}}$$

$$z = \frac{-0.009}{0.01} = -0.9$$

The p value associated with z = -0.9 is p = 0.313.

4.7 Agreement Between Detuned Testing Results and "Health at Time of Test"

At the onset of HIV reporting, clinicians were asked to classify the stage of HIV that each client presented with at the time of their first reported HIV positive test. Definitions for each classification can be found in Appendix A. Table 4.4 displays the number (%) of clients with seroconversion illness, latent (asymptomatic) HIV, later stage (symptomatic) HIV, and AIDS in the post reporting period. The majority of subjects were in the asymptomatic stage of their illness with less than 15% presenting with seroconversion illness at the time of testing.

Table 4.4 Stage of HIV According to "Health at Time of Test" (Post Reporting Period; n = 1368)

	Frequency	%
Seroconversion illness	189	13.8
Latent (asymptomatic) HIV	885	64.7
Later (symptomatic) HIV	63	4.6
AIDS	103	7.5
Unknown (clinician did not provide information	128	9.4

A Kappa statistic was calculated on the post-reporting dataset to determine the degree of agreement between the clinician's classification of seroconversion illness and the detuned test classification of RSC using only cases who had a detuned test done and whose clinician classified their health at time of test (n = 387). The results of the Kappa test was 0.126 which suggests only slight agreement between the detuned test results and the clinician HIV classification of staging. Table 4.5 displays the frequencies used for the Kappa test statistic.

Table 4.5 Frequency of Detuned Classification of RSC and Clinical Classification Used for the Kappa Test Statistic

		Detuned Test Results	
		Recent	Established
Health at Time of Test Classification	Recent	27	33
	Established	90	237

4.8 Characteristics of Individuals Undergoing HIV Testing Early in the Illness

An explanatory logistic regression model was done to explore factors associated with testing for HIV early after becoming infected (i.e. recent infection). A total of 1581 cases underwent detuned testing in the study period. According to the detuned test results, 447 (28.3%) were RSC. Table 4.6 displays the results of bivariate analysis looking for variables significantly associated with being recently infected among cases who had sufficient serum available for detuned testing.

Table 4.6 Bivariate Analysis to Detect Associations With Being RSC and Variables of Interest.

	Recent Infection n = 447 (%)	Established Infection n =1140 (%)	P value from Chi Square Test (excludes unknowns)
Ethnicity			
Caucasian	317 (73.4)	703 (66.0)	
Asian	15 (3.5)	98 (9.2)	**p = <0.001**
Aboriginal	87 (20.1)	141 (13.2)	
Other (Black/Hispanic/Mixed)	13 (3.0)	123 (11.5)	
Age			
≤ 20 yrs	18 (4.0)	23 (2.0)	
21-40	277 (62.1)	623 (55.0)	**p = 0.002**
41-60	138 (30.9)	449 (39.6)	
≥ 61 yrs	13 (2.9)	37 (3.2)	
Gender			
Female	105 (23.5)	223 (19.8)	p = 0.10
Male	342 (76.5)	906 (80.2)	
MSM			
Non-MSM	268 (60.0)	725 (63.9)	p = 0.14
MSM	179 (40.0)	409 (36.1)	
IDU			
Non-IDU	255 (57.0)	760 (67.0)	**p = <0.001**
IDU	192 (43.0)	374 (32.9)	
Sex Work			
Non-Sex	407 (91.1)	1091 (96.2)	**p = <0.001**
Worker	40 (9)	43 (3.8)	
Sex Worker			
Study Period			
Pre Reporting	311 (69.6)	800 (71)	p = <0.7
Post Reporting	136 (30.4)	334 (29)	

According to the above bivariate analysis, age, ethnicity and risk factors (binary variable: IDU, and sex work) were significantly different between groups and therefore were entered into the model. HIV subtype was not considered for the model due to the large number of missing values. Similarly, less than half of the samples were tested for primary drug resistance (36% in the pre-reporting group and 24.3% in the post reporting group) and therefore this variable was

not entered into the model. Although not significant in the bivariate analysis, gender was entered in the model to control for potential confounding as described by Sun et al.(72).

4.8.1 Collinearity

Table 4.7 displays the levels of statistical significance associated with chi-square tests conducted on independent variables are found in the table below (excludes unknowns). The results demonstrate that there are no associations between the independent variables and therefore, collinearity was not taken into consideration when constructing the model.

Table 4.7 Chi Square Analysis Conduct on Independent Variables to Detect Collinearity

	Ethnicity	**IDU**	**Sex work**	**Gender** (excludes transgendered)	**Age category**
Ethnicity		<0.001	<0.001	<0.001	<0.001
IDU	<0.001		<0.001	<0.001	<0.001
Sex work	<0.001	<0.001		<0.001	<0.001
Gender (excludes transgendered)	<0.001	<0.001	<0.001		<0.001
Age category	<0.001	<0.001	<0.001	<0.001	

Significant variables (p = <0.1) were manually entered into the model. The final model consisted of ethnicity, age, and STW and gender. Gender was not significant in the model, but was kept in to control for potential confounding. IDU was not significant in this subset of cases that were detuned tested, and therefore, was not entered into the model. The Omnibus Test of mode coefficients revealed that the goodness-of-fit of the model was good (χ^2 = 79.82; df= 8;

p=<0.001). Table 4.8 displays the crude odds ratios and the adjusted odds ratio from the multivariable logistic regression model.

Table 4.8 Crude Odds Ratios and the Adjusted Odds Ratio From the Multivariable Logistic Regression Model

	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	P Value Of variable in model
Ethnicity			
Caucasian	Reference	Reference	
Asian	0.339 (0.194, 0.594)	0.327 (0.187, 0.575)	**<.001**
Aboriginal	1.368 (1.015, 1.844)	1.153 (0.842, 1.579)	0.376
Other (Black, and Mixed)	0.234 (0.130, 0.421)	0.213 (0.117, 0.386)	**<.001**
Age			
<20	Reference	Reference	
21-40	0.568 (0.302, 1.07)	0.648 (0.331, 1.267)	0.648
41-60	0.393 (0.206, 0.749)	0.433 (0.217, 0.864)	**0.018**
>60	0.449 (0.186, 1.086)	0.480 (0.187, 1.229)	0.126
Sex Work			
No	Reference	Reference	
Yes	2.494 (1.597, 3.893)	1.784 (1.092, 2.914)	**0.021**
Gender			
Female	Reference	Reference	
Male	0.802 (0.616, 1.043)	1.056 (0.779, 1.431)	0.725

The final model shows that the odds of Asian and "Other" races being recently infected at the time of being newly diagnosed with HIV are 0.3–0.2 times the odds (respectively) of Caucasians being recently infected, all other things being equal. Similarly, the odds of a person 41-60 years old being recently infected are 0.4 times the odds as someone under the age of 20 yrs being recently infected. Finally, the odds of a sex worker being recently infected are twice the odds of a non-sex worker being recently infected all other things being equal. It should be noted that 5%-7% of the variation in the dependent variable (recent on non-recent infection) could be

explained by the predictive variables entered into the model. This suggests that the results of the model may be largely explained by random noise and caution should be taken when making inference to other populations.

CHAPTER 5: DISCUSSION

This study was successful at using a novel laboratory method to evaluate the impact of HIV Reporting on the proportion of new HIV cases testing early (within 6 months of becoming infected). Enhanced contact tracing was introduced in 2003 when HIV was added to the list of reportable diseases in BC. The desired outcome was early detection of new cases preferably at a time when cases are most infectious (i.e. during acute or recent phase). This control strategy is particularly critical for reaching individuals who are undiagnosed and asymptomatic (73). Individuals unaware of there HIV positive status are 3.5 times more likely to transmit their virus than individuals who are aware (74). Strategies aimed at increasing screening in these hidden populations can effectively reduce risk behaviour by one half (20). Reducing the time from infection to diagnosis is fundamentally important to reducing onward transmission and may help achieve the BC Ministry's of Health's goal of reducing HIV incidence by 50% over a 5 year period ending 2008 (75).

Historically, the impact of partner notification efforts on early detection has been evaluated by calculating the proportion of new cases identified from partners elicited, located, notified and tested (76). The evidence surrounding partner notification by public health suggests that it is cost effective, results in larger number of partners elicited (37), and larger numbers of partners who were unaware of their HIV positive status (77). While this evidence is impressive, methods used to evaluate partner notification efforts are highly dependent on participant cooperation and is vulnerable to recall bias and social desirability response bias. Biological makers of early detection, such as results from a detuned assay, provide an objective means of evaluating interventions.

Previous researchers have used the detuned test to evaluate HIV prevention interventions, such as needle exchange (77). While other studies have attempted to evaluate partner notification (78-80), this study is the first study, that the author is aware of, that has used a biomarker, such as detuned test results, to evaluate partner notification. B.C. is the ideal environment to evaluate the impact of HIV reporting because all HIV confirmatory testing is conducted in one central laboratory (Provincial Health Service Authority Laboratory). Newly positive cases are separated from repeat positive tests (duplicates) on a regular basis (81). Therefore, we have assurance that the dataset only includes data related to people who have become positive for the first time.

5.1 Association Between HIV Reporting and Testing Within 6 months for HIV

Our a priori hypothesis was that there would be $\geq$ 5% difference in the proportion of RSC among people testing newly positive pre-reporting compared to those testing newly positive post-reporting. While there were more than the required number of cases with detuned results in the pre-reporting group (n=1111), there was less than half the required amount (n=470) in the post reporting group. The 1% difference between groups that was detected resulted in a non significant statistical z-statistic. Additionally, the confidence intervals for the proportions of RSC in each study group over lap which indicates that the two groups are from the same population. This study was underpowered to detect a 5% difference and it is possible that a Type II error may have occurred.

5.1.1 Ad Hoc Analysis

The statistical significance of the results of our study is based on the a priori estimate of the difference in the proportion of RSC between groups for our hypothesis. Our estimate of 5% was based on the minimal amount of difference that would be clinically significant and which would impact the epidemic. If however, we hypothesized that a 7% difference was clinically significant, the sample size would have been adequate to provide 80% power at a 0.5% level of significance. Under these circumstances, we could have accepted the null hypothesis and determine that there was no statistical difference in the rate of RSC between study groups. Stated another way, if there is a difference between groups, it is likely less than 7%.

Another ad hoc analysis was done on a subset of HIV subtype B only cases. This would provide a sample of cases with the least chance of misclassification. Among these 813 cases (512 pre-reporting; 301 post-reporting) there were 159 (31%) recent infections in the pre-reporting group and 91 (30%) recent infection is the post-reporting group (p=0.81). Once again, the sample size did not provide enough power to test the significance of this 1% difference.

Finally, there was a period of transition after the date of initiation of HIV reporting when health care providers and public health nurses were learning the pre-test counselling process as outlined in the HIV Reporting guidelines. This transition in practice may have created cross-contamination of the intervention. Therefore a sensitivity analysis was conducted using a data set which excluded subjects who were diagnosed $\pm$ 6 months of the change in the program. Among these 1342 cases (987 pre-reporting; 355 post-reporting), 26.3% were recently infected at the time of being newly diagnosed in the pre-reporting group, compared to 30.7% in the post-reporting group (p=0.21). Once again, the sample size did not provide the power to test the significance of this 4% improvement and may only have occurred by chance. It is suggestive the

cross-contamination may have occurred and any future analysis should consider this method of de-selecting cases prone to cross-contamination.

5.1.2 Misclassification Bias

The detuned test, developed in 1998, has been criticized for it's propensity for misclassify established infections as recent and vice versa. Modelers justify its use by explaining that these two misclassifications cancel each other out (82). However, there is no guarantee that these misclassifications will occur in equal quantities resulting inaccurate estimates of incidence. When used to evaluate an intervention within the same population, misclassification bias is assumed to be equal between groups making comparison possible. While the study groups were different in terms age and the proportion of MSM and IDU, these characteristics are not prone to misclassification with detune testing.

Previous researchers have reported that serum from individuals who are on, or who recently finished, antiretroviral therapy will also have low antibody levels and therefore prone to misclassification error (18,83). Information about antiretroviral use was not available for this study and therefore it is possible that subjects who were on antiretroviral therapy were misclassified. However, BCCDC uses a rigorous method for determining if a positive HIV result is a "first positive" and therefore, it is very unlikely that the subjects included in this analysis were on ARVs for HIV. In addition, nucleoside reverse transcriptase inhibitors and protease inhibitors used to treat HIV are only used for HIV-1 and therefore we can say with confidence that the chance of misclassification is small.

5.1.3 Confounding

The number of HIV tests performed in a population may influence the number of true incident cases. Modelers in the UK investigated the influence of HIV testing uptake on HIV incidence among MSM and they found that, in their population, the rise in new diagnoses of HIV was attributable to increased testing uptake rather than an increase in true incidence (84). During the study period, there was a general upward trend in the number of HIV tests per quarter. A univariate linear regression model indicated that this is a statistically significant upward trend ($p < 0.001$). However, this number does not represent individual people. Therefore, it is unclear whether the increase in number represents more frequent testing in the same number of individuals or more individuals testing for HIV. The number of reported cases per year has remained constant during the study period with 401 cases in 2000 and 401 cases in 2005 with the percentage of reported cases among all HIV tests in the pre-reporting period (50.1%) equaling the percentage in the post-reporting period (49.9%). These statistics are related to the number of new cases detected each year but say little, about the number of new infections during these years. Evaluation of interventions should be conducted using true incident data such as that generated by the detuned test.

5.2 Comparison of the Proportion of Individuals Presenting With AIDS at the Time of Their First Positive HIV Test Before and After HIV Reporting

The goal of making HIV reportable was to reduce the time from infection to diagnosis. Measuring the number of people being diagnosed within six months of becoming infected is one method of evaluating this goal. However, it is hoped that the number of people newly diagnosed with HIV and concurrent diagnoses of AIDS would also be reduced as a result of enhanced

partner notification. Our study reveals that the proportion of newly diagnosed cases of HIV which received a concurrent diagnosis of AIDS (within 3 months of diagnosis) increased slightly from 6.7% in the pre-reporting group to 7.6% in the post-reporting group (p=0.313). Therefore the null hypothesis should not be rejected. The proportion of newly diagnosed cases of HIV with concurrent diagnoses of AIDS is considerably smaller than some high prevalence urban areas such as New York city (28%) (85) or more general populations in developed countries (20%) (86). This may be explained by BC's outreach programs and good uptake of HIV testing.

It is important to consider that, in rare cases, primary infection is associated with acute immunosuppression which can cause a transient AIDS defining illness, such as was the case of a male in Texas who was acutely infected (negative EIA, but positive PCR and p24 serology) with HIV with a clinical picture of cytomegalovirus (CMV) (87). This may have cause a misclassification error in the AIDS reporting database. It is unclear whether this bias exists in the BC dataset. However, if present, this bias should be consistent in both study groups and therefore should not impact the data analysis.

5.3 Degree of Agreement in Physician Classification of RSC at the Time of First Positive HIV Test and Detuned Laboratory Classification of RSC

As part of the HIV reporting documentation, care givers of newly diagnosed HIV clients are asked to classify their clients as having either acute seroconversion, asymptomatic, late HIV, or AIDS based on their clinical picture at the time of their first diagnosis of HIV. This study sought to determine the level of agreement between clinical assessments and biological markers of recent infections according to the detuned test. This information may be useful in resource poor countries where CD4 cell counts or total lymphocyte counts cannot be performed and

clinicians use the WHO recommendations of offering ART to clients who meet the WHO clinical classification of stage III or IV disease (88-89). Kagaayi et al. (90) highlighted the inaccuracies of clinical assessment in their study of 1221 HIV positive patients in Uganda which showed that by conducting a clinical assessment, clinicians could predict a CD4 call counts of 200 cells/µl or less in only half the study patients.

In our study, it would be useful to know if these to variables count be used interchangeably when modeling incidence or evaluating interventions. The Kappa score of 0.126, according to Landis and Koch (59), suggests that there is only slight agreement between the detuned test results and the clinicians assessment of HIV stage at the time of first positive. This analysis was conducted only on the post-reporting group as the method of collecting clinician assessment was more systematic after the start of HIV Reporting by using a standardized question in the reporting case report form. To display the impact of selecting this subset of data, a sensitivity analysis was done by repeating the Kappa test with both pre and post reporting groups. The Kappa score with this data set of 1391 cases remain poor at 0.101.

Misclassification bias posed a threat to internal validity of this analysis. In order to minimize this bias, cases with the highest chance of misclassification (i.e. clinician assessment of AIDS and/or non-B subtype and/or subtype unknown) were removed from the post-reporting subset of data leaving 210 cases for which the Kappa test was repeated. In this analysis, the level of agreement was reduced to a kappa score of 0.098 which is still considered poor agreement.

The reason for the lack of agreement is unclear, but it is reasonable to suggest that the lack of agreement is due, in part, to poor performance characteristics of the detuned assay. Although detuned testing methods are still widely used for estimating HIV incidence (91-94) recent researchers have questioned the utility of the Vironostika detuned assay used in this study,

especially at an individual clinical level (95), because of it's low sensitivity and specificity (96). In addition, Canadian researchers have reported that physicians are able to make a diagnosis of acute seroconversion in less than 40% of symptomatic seroconversion cases (97). With misclassification occurring in both methods of assessment, the lack of agreement measured by the Kappa statistic is understandable.

5.4 Characteristics of Individuals With RSC at the Time of Testing Newly Positive

In this analysis, logistic regression was used to model the association between demographic/risk behaviour characteristics and being RSC. This association can be interpreted as identifying sub-populations with the highest true incidence of HIV. Alternatively it can be interpreted as identifying sub-populations who are most likely to go for an HIV test within six months of being exposed to HIV.

Our results revealed that individuals who are Asian or of "other race", and/or aged 41-60 yrs of age are least likely to be recently infected at the time of first diagnosis of HIV. These results either mean that there is high prevalence among Asian middle aged men (for example) but they are delaying testing, or there is low prevalence among this sub-group. From epidemiological studies of HIV/AIDS in B.C. it seems likely that latter is true. For example, we know that very few cases of AIDS occur in middle aged Asian men. If prevalence was high but delay in testing was occurring, we would still see a high rate of reported cases of AIDS in this subgroup. Since AIDS is found rarely in Asian men it is most likely that the results of the regression analysis are indicative of low prevalence.

Conversely, sex workers were found to be twice as likely to be recently infected at the time of first diagnosis compared to non-sex workers. These findings may be suggestive of

regular testing in this sub-population. In Vancouver's downtown east side, numerous clinics servicing marginalized populations (particularly women) exist. Sex workers are accessing these clinics for various reasons and therefore are being offered HIV testing regularly. This is particularly true of indoor massage parlour sex workers (98).

Our findings are dissimilar to those of previous researchers who have reported that MSM are more likely to be recently infected than non-MSM (62,99-101). We did not find this association even after adjusting for demographics and risk factors. This is somewhat surprising considering our sample had an over representation of MSM. It is possible our results are reflective of a long history of testing in this population. However, further research should be done on the BC data to explore this difference.

Due to the small number cases that underwent drug resistance testing, we were unable to enter this variable into the logistic model. The chi-square test (Chapter 4) conducted did not reveal a positive association between recent infections and drug resistance (p=0.31). In addition, the crude odds ratio among cases who were detuned tested and drug resistant tested (n=799) was 0.90 (95% CI: 0.52, 1.56) also suggestive of no significant association. However, this is not consistent with investigations conducted by the Canadian HIV strain and drug resistance surveillance program on the same population which revealed that recently infected individuals who were newly diagnosed between 2000 and 2001 had a higher rate of primary drug resistance than the individuals who had established HIV at time of diagnosis (81). Further research should be done on this discrepancy.

It should be noted that this analysis explores associations between being recently infected and attributes of individuals. However, we know from the body of literature surrounding social networking and STI transmission, the social and sexual associations individuals have affect

testing patterns and disease transmission in the circles people travel in (102). It was not within the scope of this study to examine the effects of social networking on being recently infected when diagnosed with HIV.

5.5 Limitations

As mentioned earlier, one of the goals of HIV reporting is to reduce time from infection to diagnosis. This study did not show a reduction in the proportion of people testing within six months of infection. It is possible that the mean time from infection to diagnosis has been reduced beyond six months post infection. Since the assay used does not provide an exact time from infection to diagnosis (but merely a categorized status of recent or non-recent) reductions in mean time such as five years to three years would not be detected in this study and would be very important in terms of disease control.

There is likely considerable selection bias in the study sample. High risk individuals, such as MSM and IDU, are over represented in the sample. Theoretically, this should bias the results away from the null as it is thought that testing frequency may be greater in high risk individuals (99). This would result in an increase in incidence rates.

In addition to selection bias, the study was designed to compare one group of HIV positive cases in certain time frame to another group in another time frame. By definition, this should be considered an ecological study and therefore is vulnerable to ecological fallacy. However, since the results did not return a significant association between HIV Reporting and being RSC at first diagnosis, ecologic fallacy is not an important consideration.

Differences in demographic and risk characteristics between the two study groups raises the question about whether the groups should be compared. There was significantly more serum

available for detuned testing in the pre-reporting group (81.2%) than the post-reporting group (34.6%) (p=<0.001). Overall there is an over-representation of IDU and IDU/MSM in the study sample and an under-representation of sex workers in the study sample. Additionally, there was a higher proportion of HIV subtyping and tests for drug resistance conducted in the pre-reporting group (37.5% and 36.5% respectively) than the post-reporting group (24.5% and 21.9% respectively). These differences may have posed a threat to internal validity of the results.

Another limitation of this analysis was the amount of cases that were excluded because they did not undergo detuned testing. Dukers et al. (19) handled the problem of missing samples by repeating their analysis excluding the years with high percentages of missing samples available for their detuned testing. However, 42% of the cases in the study are missing detuned results and this proportion makes it inappropriate to exclude years with large amounts of missing data. Imputation of missing data is not recommended for the same reason.

5.6 Implications and Recommendations for HIV Programming/Policy

Due to the lack of power for this study, limitations arise in suggesting implications on HIV programming and policy. However, some insights can be gleaned from our findings that make it possible to make the following plausible suggestions:

5.6.1 HIV Reporting

When HIV Reporting was introduced in BC, many community groups expressed objections to the legislation due to fears of driving high risk individuals underground thus delaying testing. The previous evaluation conducted (24) demonstrated that HIV testing after the start of HIV reporting actually increased rather than decreased. Our research has show a slight

increase in the proportion of incident cases after HIV reporting but it is possible that these finding are a result of chance. However, we feel confident in saying that we did not find any evidence to suggest that HIV Reporting has produced impressive delays in testing. The previous evaluation reported that no incidents of harm were reported as a direct result of HIV being a reportable disease. On the other hand, numerous benefits to HIV Reporting have been suggested. When public health nurses are involved in HIV follow-up and case management, newly positive individuals gain added support in accessing HIV care (24). These suggestions are anecdotal in nature and research should be done to test this hypothesis by measuring the time from infection to access to HIV health care using viral load testing as proxy measure for access to HIV care. Overall, due to the lack of evidence suggesting negative effects of HIV Reporting, we suggest that the potential benefits warrant the continuation of HIV Reporting.

5.6.2 Detection of Incident Cases

By linking the AIDS surveillance data with the detuned test results, we were able to demonstrate a high rate of misclassification from the detuned testing algorithm. These findings are consistent with previous findings by other researchers. For this reason, we concur with previous opinions that the detuned test is not a reliable tool for identifying incident cases of HIV and therefore we recommend that other laboratory methodologies should be sought for use in modeling true incidence and prevalence in populations. Modelers have made assumptions that the percentage of recent cases misclassified as established are cancelled out by the percentage of established cases misclassified as recent. However, there is no evidence supporting this assumption and further research should be done to test this hypothesis. Alternative laboratory

tests currently available, such as the avidity test, may produce less misclassification and more accurate estimates.

Other non-laboratory methods could be employed to determine HIV prevalence. These include random sampling of the general population to HIV testing or opt-out testing which is currently being suggested in the United States. However, there are important ethical implications to these methods, including lack of personal choice and lack of informed consent associated with these methods. Therefore, we do not recommend these means of determining HIV prevalence when conducting HIV surveillance and disease control.

It is possible that, on a population level, a combination of different testing methodologies, such as the detuned test, avidity test, as well as well as other elements of HIV diagnosis such as CD4/viral load counts at time of diagnosis, may produce a better prediction of whether new cases are truly incident. Therefore, we recommend developing a validated algorithm to determine HIV incidence. We are not suggesting that this could be used for individual clinical purposes; it may produce better estimates for modeling purposes.

5.6.3 Use of the Detuned Test

Due to the poor proficiency of the detuned test, we are also not recommended the detuned test for use on an individual basis. At a population level, we have demonstrated that it is a satisfactory laboratory tool for evaluation purposes, especially when comparing an outcome before and after a public health intervention, such as enhanced partner notification. This is because any bias introduced as a result of poor proficiency is assumed to be equal over time. In this respect, the value of the detuned test is equal to other laboratory tests available for identifying incident cases and therefore, we recommend it's use in future evaluation research.

However, the detuned test kits have been manufactured by different companies over time resulting in different window periods and different thresholds for determining "recent infections." These factors must be considered when interpreting detuned results over time.

5.7 Future Research

Innovative methods of conducting partner notification, such as internet based notification, have emerged in the past decade which enhance traditional notification practices (103). The methods used in this study could be used to evaluate the impact of these novel partner notification practices. Lessons learned from this study, have highlighted the need to do prospective testing for RSC rather than depending on looking retrospectively at testing conducted on "left over" serum. Prospective collection of the data would minimize selection bias and improve internal validity.

5.8 Summary

A key element of controlling any communicable disease, including HIV, is early detection. Recently infected individuals will likely change risk behaviour earlier in their illness and therefore expose fewer people to the virus. Interventions, such as enhanced partner notification, aimed at reducing onward transmission, are in place but the current methods for evaluating these methods are vulnerable to reporting bias. This study has effectively used a biological marker that enabled an objective comparison of partner notification by comparing the proportion of RSC before and after the introduction of enhanced partner notification used with HIV Reporting. Future research, including a larger sample size, could be done to support our

hypothesis generating findings. It is also recommended that such research include a laboratory method with improved test characteristics such as the avidity test.

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APPENDICES

Appendix A: Classification of HIV Staging on BC HIV Case Report Form

Seroconversion illness:	Person experienced an acute mononucleosis-like illness/ severe flu-like illness (fever, sweats, aching joints, weakness) sever flue like symptoms (fever, sweats, aching joints, weakness)
Asymptomatic (Early stage HIV)	Newly diagnosed person feels well. No symptoms of seroconversion/ no early symptoms of HIV disease
Symptomatic (Later stage HIV)	Person has lingering illnesses but has not had an opportunistic infection which would infer a diagnosis of AIDS
AIDS	Patient has been diagnosed by a physician as having one or more of the opportunistic infections described by Health Canada. When a person has a diagnosis of AIDS, and AIDS Case Report form should be completed and submitted to public health.
Unknown.	Healthcare provider has little or no awareness of client's state of health as regards to HIV.

Appendix B: Modified Canadian AIDS Case Definition

Laboratory Data

- Laboratory evidence of HIV infection in persons over 15 months of age or in non maternal-fetal transmission

For the purpose of surveillance, a confirmed, repeatedly reactive screening test for HIV antibody constitutes sufficient laboratory evidence of HIV infection in any person over 15 months of age or in infants less than 15 months of age if maternal-fetal transmission is not suspected. Other acceptable evidence is outlined in the Canadian Disease Weekly Report 1987;13-38:169-176 and the Canada Communicable Disease Report 1993;19-15:116-117.

- In the absence of laboratory evidence of HIV Infection, causes of Immunodeficiency that DISQUALIFY diseases as indicators of AIDS are:

1. High-dose or long-term systemic corticosteroid therapy or other immunosuppressive/cytotoxic therapy within 3 months before the onset of the indicator disease.
2. Any of the following diseases diagnosed <3months after diagnosis of the indicator disease: Hodgkin's disease, non-Hodgkin's disease, non-Hodgkin's lymphoma (other than primary brain lymphoma), lymphocytic leukemia, multiple myeloma, any other cancer of lymphoreticular tissue, angioimmunoblastic lymphadenopathey.
3. A genetic (congenital) immunodeficiency syndrome or an acquired immunodeficient syndrome atypical of HIV infection, such as one involving hypogammaglobulinemia.

Diseases Indicative of AIDS

1. Bacterial pneumonia, recurrent
2. Candidiasis (bronchi, trachea or lungs)
3. Candidiasis (esophageal)
4. Cervical cancer, invasive
5. Coccidioidomycosis (disseminated or extrapulmonay)

   Cryptococcosis (extrapulmonary)
6. Cryptosporidlosis (chronic intestinal, 1 mo, duration)
7. Cytomegalovirus disease (other than in liver, spleen or nodes)
8. Cytomegalovirus retinitis (with loss of vision)
9. Encephalopathy, HIV-related (dementia)
10. Herpes simplex: chronic ulcer(s) (>1 mo. Duration) or bronchitis, pneumonitis or esophagitis

    Histoplasmosis (disseminated or extrapulmonary)
11. Isosporiasis, chronic interstitial (>1 mo. duration)
12. Kaposi's sarcoma
13. Lymphoma, Burkitt's (or equivalent term)

    Lymphoma, immunoblastic (or equivalent term)

    Lymphoma, primary in brain
14. Mycobacterium avium complex or M. kansasil (disseminated or extrapulmonary)

    Mycobacterium of other species or unidentified species.
15. M. tuberculosis (disseminated or extrapulmonary)

16. M. tuberculosis (pulmonary)

17. Pneumocystis carinii pneumonia

18. Progressive multifocal leukoencephalopathy

19. Salmonella septicemia, recurrent

20. Toxoplasmosis of brain

21. Wasting syndrome due to HIV

Appendix C: CREB Certificate of Approval

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF EXPEDITED APPROVAL: RENEWAL

PRINCIPAL INVESTIGATOR:	DEPARTMENT:	UBC CREB NUMBER:
David M. Patrick	UBC/Medicine, Faculty of/Health Care & Epidemiology	H06-70132

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:
N/A
Other locations where the research will be conducted:
N/A

CO-INVESTIGATOR(S):
Mel Krajden
Darlene Taylor
Gina Ogilvie
Mark W. Tyndall

SPONSORING AGENCIES:
BC Ministry of Health - "Recent HIV Seroconversion at Time of First Positive Test: A Comparison Before and After HIV Reportability"

PROJECT TITLE:
Recent HIV Seroconversion at Time of First Positive Test: A Comparison Before and After HIV Reportability

EXPIRY DATE OF THIS APPROVAL: March 5, 2008

APPROVAL DATE: March 5, 2007

CERTIFICATION:
In respect of clinical trials:
1. 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.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

Approval of the Clinical Research Ethics Board by one of:

Dr. Bonita Sawatzky, Associate Chair