An international study to characterise recently acquired HIV infection in Estonia, Poland, and Ukraine

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Declaration

I, Ruth Deborah Simmons, confirm that the work presented in this thesis is my own. Where information is derived from other sources, I confirm that this has been indicated in the thesis.

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And finally, thank you to my parents for always being positive and not making their shock at my achievement too obvious and also my husband Mark, for whom the most important acknowledgement goes, thank you for believing in me.

Abstract

Serological methods to differentiate recent from non-recent HIV infections were introduced to routine surveillance in Poland, Estonia and Ukraine, to estimate HIV incidence and to characterise those newly-diagnosed and infected. Establishing the characteristics of populations at greatest risk of HIV enables appropriate and target prevention and intervention strategies to be developed, reducing risk of onward transmission.

Using existing testing services within each country, residual samples from newly-diagnosed persons were tested for evidence of recent infection using an avidity assay. Data were collated in 2013-2014 using modified existing methods in Kiev City, Poland and Estonia.

Diagnosis rates for Kiev City, Poland and Estonia were 21.5, 1.2 and 29.7 per 100,000 population, respectively. Incidence estimates for Kiev City were 21.5 per 100,000 population, with 6.5% classified as recent. The disproportionate distribution of HIV were among men who have sex with men (MSM) and persons who inject drugs (PWID) was evident. Uncorrected estimates for Poland and Estonia were 30% and 44%, respectively.

This work enables targeted public health action and health promotion work to be made, laying the foundation for local and national guidelines.

Publications Arising from this Work

Smith R, Semenenko I, Toplina M, Tereschenko R, Kotlik L, Zasyptka L, et al. High percentage of recent HIV infection leading to onward transmission in Odessa, Ukraine associated with young adults. XIX International AIDS Conference (2012).

Simmons R, Semenenko I, Toplina M, Tereschenko R, Kotlik L, Zasyptka L, et al. High percentage of recent HIV infection leading to onward transmission in Odessa, Ukraine associated with young adults. AIDS and Behavior 2013.

Simmons R, Lutsar I, Malyuta R, Rosinska M, and Porter K. Using incidence assays within the context of the Recent Infections Testing Algorithm (RITA). AIDS 2014.

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Glossary

3TC	Lumiviudine
AI	Avidity Index
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral therapy
ARV	Antiretrovirals
AZT	Zidovudine
CASCADE	Concerted Action on SeroConversion to AIDS and Death in Europe
CDC	Centre for Disease Control
CEPHIA	Consortium for the Evaluation and Performance of HIV Incidence Assays
CoV	Coefficient of Variation
CROI	Conference on Retroviral and Opportunistic Infections
CSW	Commercial Sex Workers
CVIL	Central Virology and Immunology Laboratory
ddI	Didanosine
EC	European Comission
ECDC	European Centre for Disease Prevention and Control
EFV	Efavirenz
EIA	Enzyme Immunoassay
EMIS	European MSM internet survey
EPP	Estimation and Projection Package
EU	European Union
FRR	False Recent Rate
FSW	Female Sex Workers

FTC	Emtricitabine
HAART	High Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IAS	International AIDS Society
IDE	immunodominant eptitope
IgG	immunoglobulin G
IgM	immunoglobulin M
IQR	Inter Quartile Range
IN	Integrase
ITKH	East-Tallinn Central Hospital
IVKH	IDA-VIRU Central Hospital
JSM	Joint Statistical meeting
LAg	Limiting Antigen
LMM	Linear Mixed Model
LS-EIA	Sensitive/Less sensitive enzyme immunoassay
LTKH	West-Tallinn Central Hospital
MAA	Multiassay Algorithm
MARP	Most at risk populations
MAT	Medication-assisted treatment
MDRI	Mean Duration of Recent Infection
MeSH	Medical Subject Headings
MOT	mode of transmission
MRC	Medical Research Council
MSM	Men who have sex with men
MTCT	Mother to Child Transmission
NGO	Non-Govenment Organisation
NIZP-PZH	National Institute of Public Health - National Institute of Hygiene
NLMM	Non-Linear Mixed Model
NNRTI	non-nucleoside reverse transcriptase inhibitors
NRTI	nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
OD	Optical Density

OR	Odds Ratio
PI	protease inhibitors
PPAI	Perinatal Prevention of AIDS Initiative
PWID	Persons who inject drugs
pyrs	person years
RITA	Recent Infection Testing Algorithm
SATUK	Tartu University
SEP	Syringe exchange programmes
STARHS	Serological Testing Algorithms for HIV Seroconversion
STIs	Sexually Transmitted Infections
TDR	Transmitted Drug Resistance
TIAB	Title and Abstract
TRI	Test for recent infection
UCL	University College London
UCLH	University College London Hospital
UNAIDS	Joint United Nations Programme on HIV/AIDS
VCT	Voluntary, Counselling and Testing
VL	Viral Load
WHO	World Health Organisation

Chapter 1

Characterising the Population

The following chapter describes Human Immunodeficiency Virus (HIV) in Europe and the epidemics within the three countries of focus Ukraine, Estonia and Poland. I describe the distribution of those newly diagnosed using published literature, and how the epidemic has changed over time.

1.1 Introduction

The HIV is a retrovirus which attacks an individual's immune system leaving them vulnerable to opportunistic infections and cancers which are collectively known as Acquired Immunodeficiency Syndrome (AIDS). The first diagnosis of AIDS was reported in San Francisco in 1981, and since then the number of people living with HIV world-wide is estimated to have reached an 33.3 million [31.4-35.3 million] in 2009 [1]. While there is no cure for HIV, the virus can be managed using combinations of Antiretroviral (ARV) drugs. These drugs introduced in the mid-1990s have increased the life expectancy of HIV infected individuals, resulting in a decrease in the number of AIDS diagnoses and mortality. Individuals who are diagnosed early with their infection and adhere to treatment can expect a life expectancy close to that of a negative individual. Although treatment is effective, there are still a number of challenges to face, the development of drug resistance to certain treatment regimes, the lifetime cost of an individual with HIV and early diagnosis ensuring individuals can experience the full benefit of treatment. Transmission of HIV can occur through sexual contact (vaginal or anal), vertical transmission (from mother to child), injecting drugs or through contact with contaminated blood. The distributions of these transmission routes differ considerably from country to country.

The ability to estimate HIV incidence is of major public health importance, establishing current transmission patterns and monitoring prevention efforts. Data collected on testing and new diagnoses by many countries through national surveillance systems do not have the ability to determine whether increases to the HIV epidemic are due to onward transmission or increased testing of previously undiagnosed infections. Eastern Europe is an area of particular interest, where rates of diagnosed HIV have increased dramatically in recent years, and relatively little is known about each epidemic.

1.2 HIV Epidemic in Europe

Thirty years since the first AIDS diagnosis, HIV is still a communicable disease of major public health importance in Europe [2]. With no indication of a decline in new HIV diagnoses and continued growing numbers of persons living with HIV, it is imperative to build an enhanced picture of the current epidemic, whilst monitoring and evaluating new and current public health initiatives. Until recent years, European figures for HIV and AIDS have been primarily based on numbers of newly diagnosed persons with HIV or AIDS alongside those accessing HIV-related care services. This prevalent pool, although important to quantify is not necessarily reflective of the current situation given the long latent period which characterises HIV, meaning a person may be infected for many years before a diagnosis is made. Determining the number of persons who have been recently infected with HIV in a defined period enable current transmission patterns to be ascertained, highlighting populations at greatest risk, and guiding prevention and intervention strategies.

Methodologies for identifying whether an infection is recent have previously been achieved through observational studies following a cohort of sero-negative persons overtime screening for new infections or using surveillance data, through serial sero-surveys, back-calculations or other mathematical models. However, these methods can be expensive to conduct, raise questions regarding generalisability and rely heavily on epidemiological data which may or may not be collected through country specific surveillance [3]. In recent years, serological tests which identify biological markers indicative of a recent infection have been introduced. These serological tests supported by clinical evidence of either a recent or established infection enable recent infections to be monitored, calculating the proportion of diagnoses newly infected or incidence estimates for the population. This methodology is known as the Recent Infection Testing Algorithm (RITA) [4, 5].

Until the mid-1990s the HIV epidemic experienced in Europe was led predominately by Western Europe and, in particular, the 15 Western European countries of the European Union (EU) [6]. On average, 23,000 AIDS diagnoses were reported per year between 1992 and 1995 in Western Europe, 29 times higher than the 800 reported in Central Europe and 383 times higher than the 60 in Eastern Europe [6]. However, from the mid-1990s, this distribution changed dramatically, and within a short period of time Eastern Europe became the leading European

region with rapid increases in both the number of new HIV and AIDS diagnoses. Changes to the political, social and economic structure of Eastern Europe after the collapse of the Soviet Union in 1991 were influential in driving forward the HIV epidemic, with increases in illicit drug use, prostitution and rates of Sexually Transmitted Infections (STIs) [7–9]. During the ensuing seven years the diagnosis rate of HIV in Eastern Europe increased from 0.61 per 100,000 population in 1995 to 34.7 per 100,000 in 2001. HIV diagnosis rate in 2001 was six times higher than the 5.5 per 100,000 population in Western Europe [6]. Unlike the east and west regions of Europe the HIV diagnosis rate in Central Europe during this time period remained relatively low and stable, although with significant variation between countries [7].

By the end of 2010, Eastern Europe was still experiencing the highest overall rates of HIV in Europe with 31.7 cases per 100,000 population, considerably higher than that of Western Europe (6.6 cases per 100,000) and Central Europe (1.3 cases per 100,000) [10], and higher than the 18.9 per 100,000 in 2009 [11]. Importantly, in 2009, the Ukraine, Estonia alongside Russia were identified as having HIV diagnostic rates greater than 20 cases per 100,000 population.

Unlike Western Europe, where the risk of HIV was primarily among Men who have sex with men (MSM) and sex between men and women, the increasing rates of HIV in Eastern Europe were dominated by injecting drug use. As the rates of injecting drug use increased across Eastern Europe so did HIV prevalence. The first outbreaks of HIV among PWID were identified in the Ukraine, in the southern regions of Odessa and Nikolayev, preceded by outbreaks in the Russian regions of Kaliningrad, Irlutsk, Rostov-on-Don and Moscow and Sretlogorsk in Belarus [7, 12, 13]. Increases in HIV diagnoses continued, and new HIV diagnoses were detected in all regions across Ukraine, Russia, Latvia and Estonia, each predominately due to injecting drug use [9]. Central Europe experienced a very heterogeneous spread of HIV, dependent on the country affected [11]. Although, the transmission routes in Central Europe were predominately reported as either sex between men or between men and women like Western Europe, Poland bore similarities to the Ukraine and Belarus with injecting drug use being the leading route of infection [7, 9].

Injecting drug use is still a predominate factor in Eastern Europe and areas of central Europe, and it is estimated that of a population of 3,400,000 PWID in Eastern Europe, in 2007, over a

quarter (27%; 940,000) were estimated to be HIV positive [14]. Among those diagnosed with HIV in Eastern Europe during 2010, 43% were PWID, much higher than the 4% reported for Western and Central Europe [10]. Alongside PWID, numbers of HIV infections attributable to sex between men and women have also risen, suggesting a shift in transmission from PWID to their sexual partners. In Eastern Europe 48% of diagnoses were attributable to sex between men and women [10]. Data for Central Europe, indicate that 24% of diagnoses were due to sex between men and women, 29% were among MSM, 4% among PWID and the remainder (43%) with an unknown risk group [10]. It is not possible to understand the possible contribution to the epidemic of those risk groups with such a high proportion unknown.

To understand what is pushing forward the recent increase in HIV infections in Eastern Europe, the European Comission (EC) funded network of excellence, EuroCoord, made identifying and characterising new HIV diagnoses and recent HIV infection in three countries in Eastern and Central Europe: Poland, Estonia and Ukraine a priority.

This integrated network is formed from the biggest HIV cohorts and collaborations in Europe, CASCADE, COHERE, EuroSIDA and PENTA. These cohorts include seroconverters, adult, paediatric and mother/child cohorts, patients attending for care and children and pregnant women enrolled in trials and observational studies. The diversity of these cohorts enables EuroCoord to address key areas of HIV research; characterising populations in Europe, improving management of those living with HIV, and exploring specific subgroups. By investigating Eastern and Central European countries included within EuroCoord, enables populations at greatest risk to be identified, and the correct intervention and prevention methods to be adopted.

1.3 Countries and Resources: Ukraine

In 2012 the Ukraine had one of the highest rates of diagnosed HIV in Europe with 37.1 per 100,000 population [15] (figure 1.1) and an adult (15-49yrs) prevalence of 1.1% (95% CI 1.0-1.3) [1]. The first HIV diagnosis was recorded in 1987 and, between 1987 and 2012, 187,316 persons were diagnosed with HIV [15]. Cumulative figures for AIDS diagnoses 1987 to 2009 were 31,241 AIDS diagnoses and 17,791 AIDS-related deaths [16]. The majority of the diagnoses in Ukraine occurred from 1995 onwards [16] with the number of HIV diagnoses in 2009 more than double that in 2001 [1]. Rates of diagnosed HIV rose from 12.5 per 100,000 population in 2001 [10] to 37.1 in 2012 [15] (figure 1.1). It is estimated that only 28% of persons living with HIV in the Ukraine are aware of their status, equating to an estimated 360,000 persons [16].

Testing for HIV is accessible nationwide, with service initiated screening also available in antenatal settings, prisons, blood donor facilities and for policy and regulation reasons. In 2012 just over 2 million HIV tests were reported to have been performed, a 49% increase on the 1.5 million reported in 2004 [10, 15] (figure 1.1). However, even with increased testing there are still barriers, including lack of knowledge about risk, geographical barriers as individuals elect to travel to the city due to limited services outside it and their wish to maintain anonymity. stigma, and the barriers faced when identifying as an injecting drug user, a sex worker or being in a same-sex relationship [17, 18].

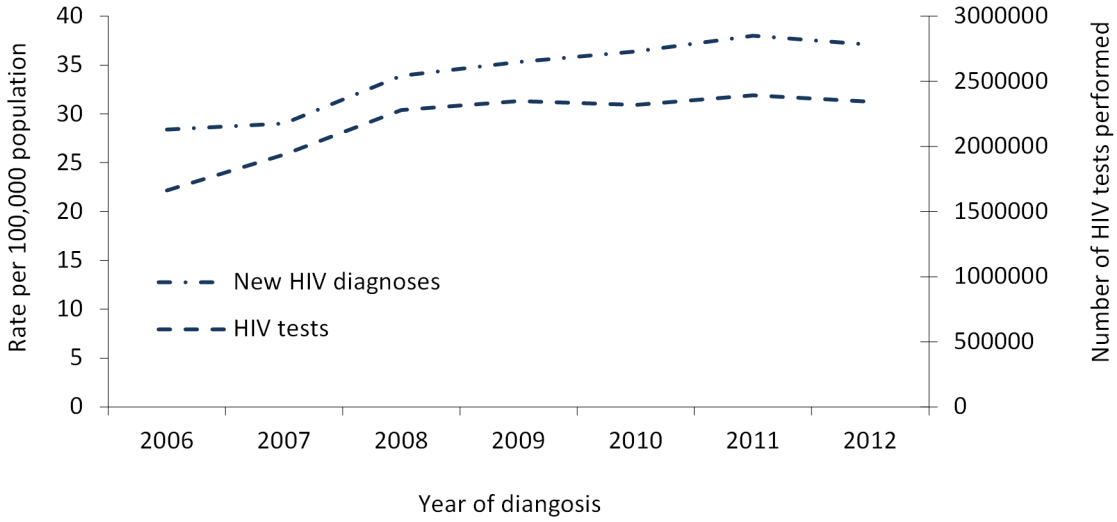


Figure 1.1: Rate of new HIV diagnoses and number of HIV tests performed, Ukraine: 2001-2010. Source: European Centre for Disease Prevention and Control [15].

There is little information about the overall testing frequencies in Ukraine, however UNAIDS reported that in 2009 59% of sex workers, 31% of MSM and 70% of PWID had had an HIV test in the last 12 months and knew the result [1]. A study monitoring the behaviour of 3,711 PWID in Ukraine identified that only half had ever had an HIV test, of whom half again had tested within the past 12 months [19]. In relation to all PWID participating within the survey this resulted in 29% of PWID seeking an HIV test within the past 12 months. Interestingly, testing for HIV was found to be linked to seeking medical attention, with 76% of PWID who had tested for HIV had also sought medical attention; among those who had never tested for HIV this was 44%. PWID who were identified as positive for HIV, 16% reported their previous test as negative and 39% had never had a previous test or were unaware of the result [19].

Although numbers of MSM are low compared to other European countries there is still a need to monitor this subgroup and understand testing behaviours. A study investigating MSM in Ukraine identified that the proportion having an HIV test in the past 12 months had increased from 28% in 2007 to 42% in 2009. Among MSM who reported their last HIV test as negative, 5% tested positive using rapid testing [20].

Testing for HIV should be sought at regular intervals among Female Sex Workers (FSW), however among those surveyed only 57% had tested within the past 12 months and knew their result. Further investigation suggested their reasons for not testing were due to lack of knowledge regarding where to test and the necessity of regular testing alongside fear of the result [21].

The leading route of infection until recent years has been injecting drug use, which was instrumental in the rapid increase in the number of new HIV diagnoses. The first outbreaks of HIV among PWID within Ukraine were in the southern regions of Odessa and Mykolaiv [7, 12, 13], and in more recent years there has been an increase in HIV rates from sexual transmission. Predominately this increase is reported to be due to sex between men and women, however due to stigma which surrounds same-sex transmission it is unknown how much MSM is also a contributing factor [16, 22]. It is postulated that the 94 MSM reported in 2009 is a considerable underestimation, with the prevalence of surveyed MSM 8.6% [20]. Most at risk populations for Ukraine are PWID, FSW and MSM, with a sentinel epidemiological study estimating the proportion infected with HIV in each population to be 21.5%, 9.0%, and 6.4% respectively.

In addition, in 2008, the number of diagnoses reported to be due to sex between men and women crossed that of persons infected through injecting drug use [16], suggesting a shift in transmission from most-at-risk populations to the general public [23] (figure 1.2). However, the sero-prevalence of HIV among PWID in 16 Ukrainian cities in 2008 remained high at 32% [24]. In addition to this shift is the increasing number of new diagnoses among women, with recent figures suggesting that women account for almost half of those diagnosed. It has been suggested that this shift in the epidemic is predominantly due to women who inject drugs being at higher risk as they are more likely to be second on the needle, to be commercial sex workers exchanging sex for drugs, subject to gender-based violence and stigma, or through unsafe sex with an injecting partner [25–27].

Mother to Child Transmission (MTCT) in the Ukraine is the leading route of infection among children, resulting in the introduction of the national MTCT programme for preventing HIV in newborns in 2001 [28,29]. The outcome has been a decrease in rates of MTCT from an estimated 27.5% in 2000 [28] to 7.0% in 2006 [29]. However, even with these successes, the uptake of antenatal services among hard to reach populations such as female PWID is low [30].

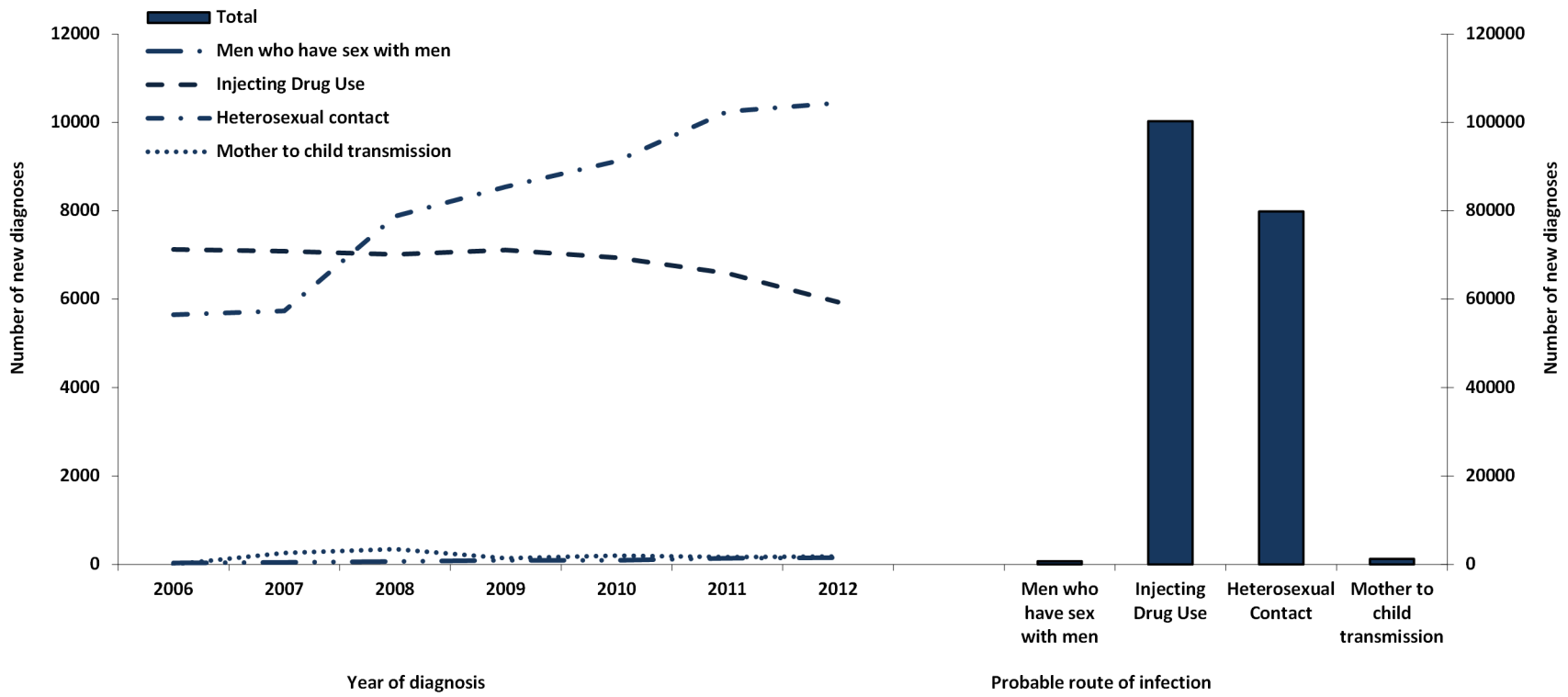


Figure 1.2: Number of new diagnoses by probable route of exposure; line graph represents the number of new diagnoses by probable route of exposure and year of diagnosis; Bar graph represents the number of cumulative diagnoses by probable route of exposure: 1987-2010. Source: European Centre for Disease Prevention and Control [15].

The predominant subtype for the Ukraine has been identified as A, followed by a smaller proportion of subtype B [31,32]. Of 163 specimens from HIV infected patients sequenced in 2006, 66% were classified as subtype A and 30% as subtype B, other subtypes identified were C (2%), D (1%) and a unique A/B recombinant (1%) [33]. The formation of these subtypes followed the initial outbreak of PWID, with transmission by subtype A occurring within the Odessa outbreak and subtype B in Nikolaev region. Following the outbreaks, subtype A subsequently spread to other former Soviet union states, whereas subtype B did not [34]. However, as a result of both subtypes being active within the Ukrainian population and in neighbouring countries, a small proportion (1%) of infected individuals with a recombinant A/B subtype CRF03_AB has been identified [33, 35].

1.3.1 Odessa

Overall figures at the end of 2009 suggest that 11,204 individuals are infected with HIV in the Odessa region with an HIV prevalence of 470,7 per 100,000 population [36]. These figures suggest that of the 101,000 individuals infected in the Ukraine in 2009 one in ten originated from Odessa. Odessa had the third highest number of HIV infections after Donetsk and Dnipropetrovsk regions. Population estimates of at risk groups most vulnerable to HIV for 2009 were 40,000 PWID, 8,000 MSM and 5,500 FSW [36].

Following outbreaks of HIV among PWID in 1995, Odessa has contributed to the high numbers of HIV diagnoses in Ukraine. The rapid increase in HIV prevalence of PWID following 1995 is illustrated in figure 1.3 using a mathematical stimulation model [37] with the prevalence in 2009 estimated to be 53% [38]. The mathematical stimulation model uses data available on PWID population, PWID mortality rate, mean duration spent as an PWID, demographic distribution of PWID, risk behaviour indicative of injecting drugs, sexual behaviour and HIV transmission rate by behaviour risk.

Strathdee *et al* also projected the number of new HIV infections among PWID in Odessa, assuming no change in risk. This figure is estimated to be 6200 (95% CI: 4500-6500) new infections between 2010 and 2015 (figure 1.4). Attributable risks for new HIV infections indicated by Strathdee *et al* include inadequate Antiretroviral therapy (ART) access by those already diagnosed and non-sterile equipment use [37], the proportion of PWID using unsterilized equipment in Odessa is estimated to be between 10 and 20% [19].

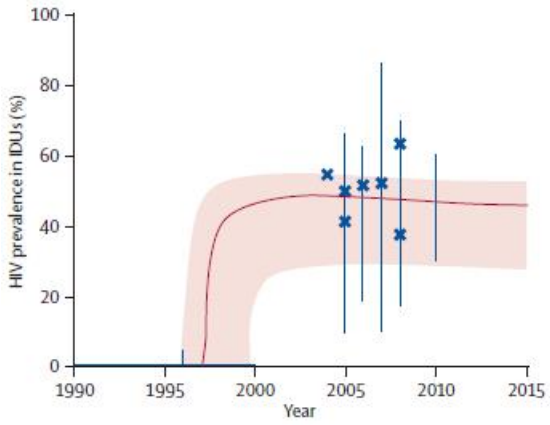


Figure 1.3: HIV prevalence among PWID, 1990-2015. Blue crosses show prevalence estimates from data; the pink area shows envelope of posterior model fits; and the thick red line shows the epidemic projection for the best-fitting model.

Source: Strathdee *et al Lancet 2010.* [37].

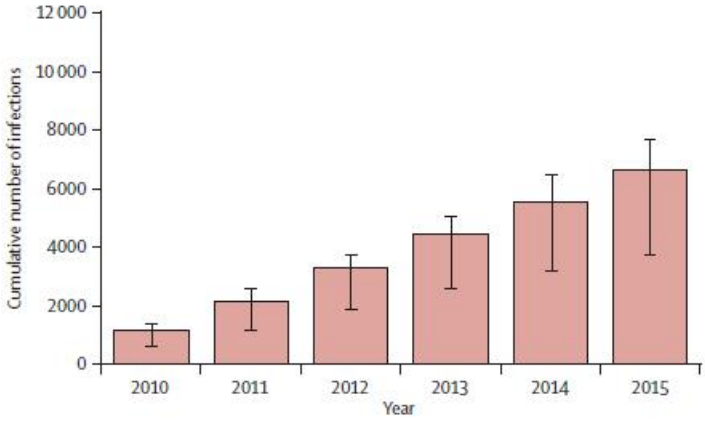


Figure 1.4: Projected number of HIV infections every year for 2010-15 in the PWID population, assuming no further changes in patterns of risk.

Source: Strathdee *et al Lancet 2010.* [37].

1.3.2 Kiev

Overall figures at the end of 2009 suggest that 6,847 individuals are infected with HIV in Kiev city with an HIV prevalence of 249,8 per 100,000 population [36]. Of the 26 regions within the Ukraine five, including Kiev City, accounted for 69% of all HIV infections. Kiev city had the fourth highest number of HIV infections after Odessa, Donetsk and Dnipropetrovsk. Population estimates of at risk groups most vulnerable to HIV for 2009 were 38,000 PWID, 14,000 MSM and 6,800 FSW [36].

1.4 Countries and Resources: Poland

Poland has a relatively stable epidemic [9, 39] and since the first diagnosis in 1985, the reported cumulative number of persons newly diagnosed in Poland by the end of 2010 was 16,562, including 2,907 AIDS diagnoses and 1,206 AIDS-related deaths [15]. The rate of diagnosed HIV for Poland was higher than the overall rate for central Europe in 2012, 2.8 vs. 1.9 per 100,000 population respectively [15] and a small increase from the 1.8 for Poland in 2004 [10] (figure 1.5). The adult (15-49) prevalence for Poland is 0.1% (95% CI 0.1-0.1) [1]. It is estimated that a third of HIV positive individuals in Poland are unaware of their HIV status, with the number of individuals living with HIV estimated to be as high as 30,000-35,000 [40, 41].

HIV testing is available in Poland through medical, service initiated screening and patient initiated facilities, such as Voluntary, Counselling and Testing (VCT) sites. Screening for HIV is conducted at 300 laboratories across Poland and confirmatory testing is available at 20 laboratories, the majority of which use western blot. Overall, within a year, around a million HIV tests are conducted through blood blanks and a further 200-300,000 test through all other facilities, testing information collected by European Centre for Disease Prevention and Control (ECDC) shows an increase in the number of HIV tests over the past 7 years (figure 1.5) [15]. Although a large proportion of tests are conducted via blood banks, the large majority of positive results are identified through diagnostic services or centres offering anonymous testing [40].

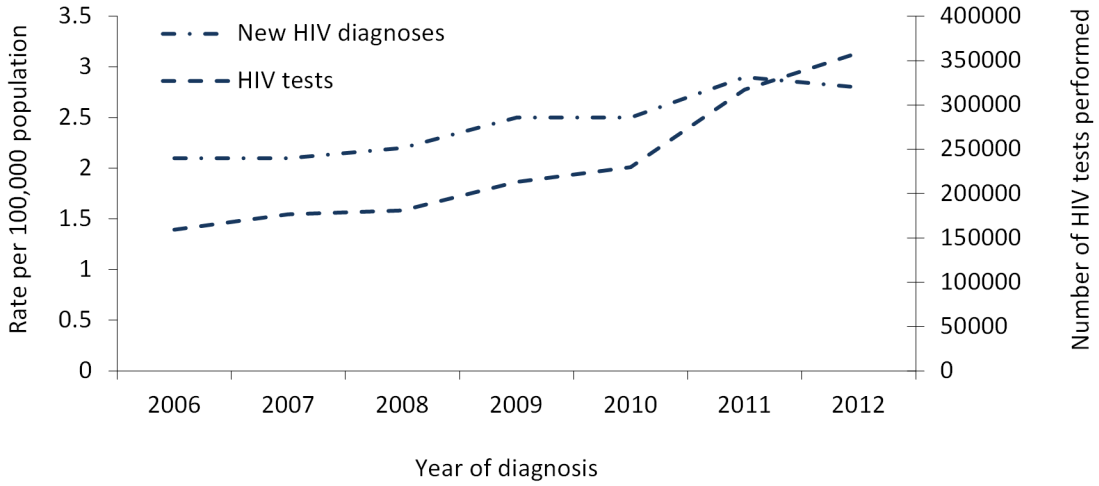


Figure 1.5: Rate of new HIV diagnoses and number of HIV tests performed, Poland: 2004-2010. Source: European Centre for Disease Prevention and Control [15].

The distribution of persons newly diagnosed has changed over time with the primary route of infection moving from PWID who, until recently, have accounted for 70% of all new diagnoses, to sexual contact [10, 42]. In a cross-sectional study of PWID entering a treatment program in Warsaw between January and September 1993 the sero-prevalence was recorded as 45.9% [43]. This concentrated epidemic among PWID was largely due to high risk behaviours such as needle sharing and a lack of needle exchange programme [43, 44], among those in the cross-sectional study, 24% reported borrowing and 37% reported passing on used syringes [43]. A needle exchange programme for Poland was initiated in 1991 [41]. Other high risk behaviour reported was sexual risk and among those PWID, who reported sexual contact (72%), 62% reported never using a condom [45].

During 2000 - 2010 the number of new diagnoses attributable to injecting drug use fell by 90%, and the number of diagnoses among MSM, increased by 345% and among heterosexual men and women, by 114% [10, 39, 40]. The number of diagnoses due to sexual contact, both sex between men and women and MSM, overtook that of persons infected through injecting drug use in 2008 [10] (figure 1.6).

There is considerable geographical variation across Poland, figures for 2010 ranged from 0.16 per 100,000 population in Swietokrzyskie to 4.55 per 100,000 population in Dolnoslaskie (figure 1.7) [46].

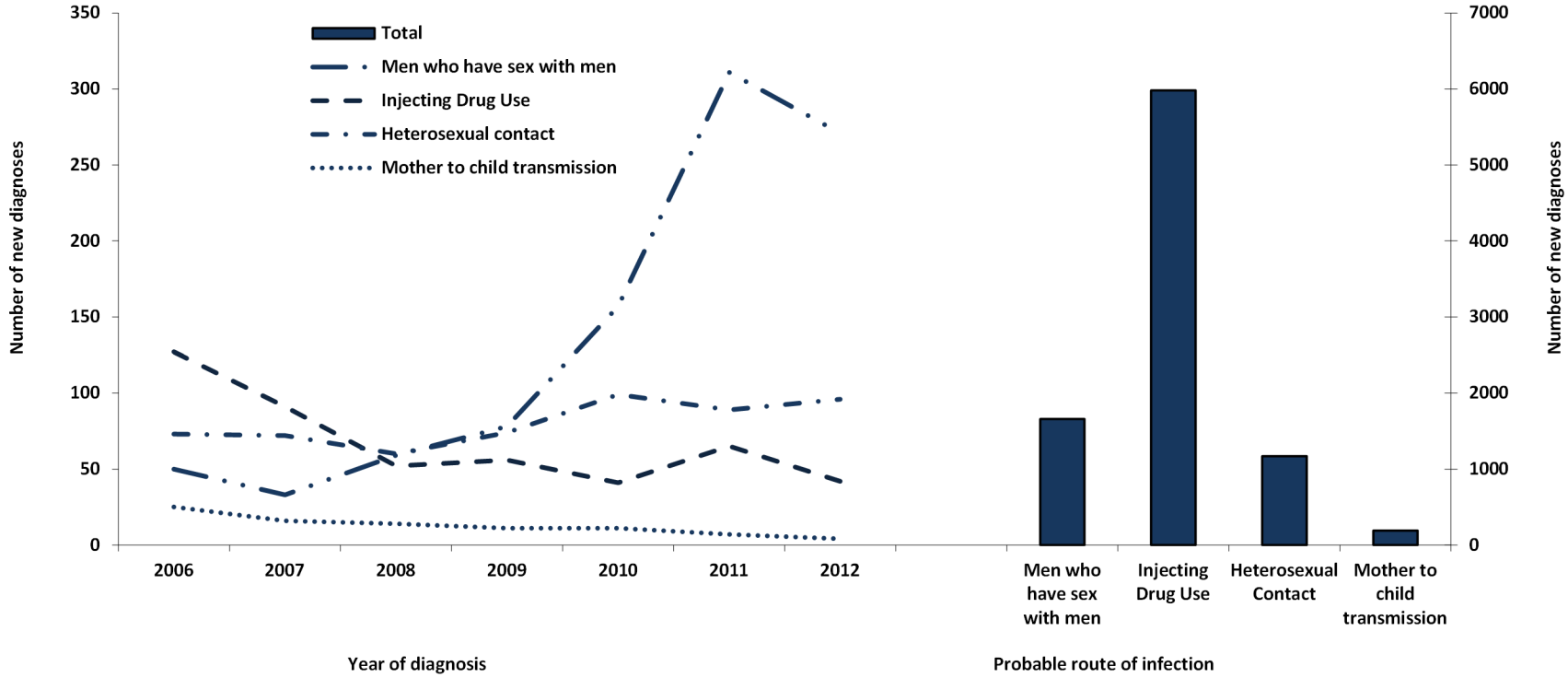


Figure 1.6: Number of new diagnoses by probable route of exposure; line graph represents the number of new diagnoses by probable route of exposure and year of diagnosis; Bar graph represents the number of cumulative diagnoses by probable route of exposure: 1985-2010. Source: European Centre for Disease Prevention and Control [15].

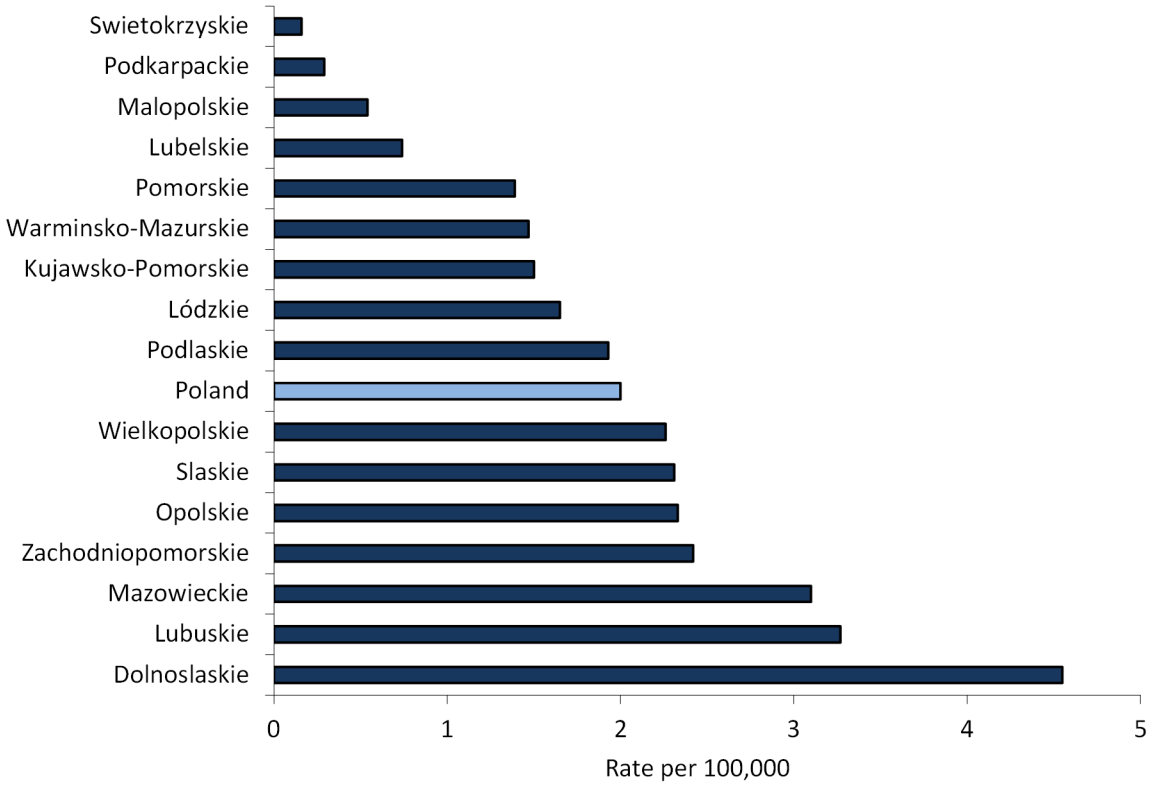


Figure 1.7: Rate of HIV infections by geographical region per 100,000 population: Poland 2010.

Source: National Institute of Public Health - National Institute of Hygiene [46].

1.5 Countries and resources: Estonia

Estonia has a relatively new epidemic, although the first case of HIV was identified in 1988, the concentrated epidemic rose rapidly during 2000 reaching the highest HIV prevalence (105.3 per 100,000 population) in the EU in 2001 [47]. Since then the incidence has been gradually decreasing but diagnoses still remains high at 23.5 per 100,000 population in 2010 [15] (figure 1.8), and was identified as one of three European countries presenting with an HIV rate more than 20.0 cases per 100,000 population [15]. Estonia is still one of the three countries in Eastern Europe and Central Asia in which an estimated HIV prevalence exceeding 1% of the adult population (15-49 years), with 1.2% [1.0-1.5] [1]. By the end of 2010 the cumulative number of new HIV diagnoses was 8377, with 390 AIDS diagnoses and 104 deaths among individuals with AIDS [15].

Testing for HIV is accessible nationwide, with service initiated screening available in antenatal settings, prisons and blood donor facilities. In 2011, over 200,000 tests were conducted on 147,000 individuals. The majority of tests (57%) were conducted in Tallinn, followed by Tartumaa (29%) [Lutsar, Personal correspondence]. Studies among high-risk populations investigating the uptake of HIV testing identified that 32% of MSM [48], 35% of Commercial Sex Workers (CSWs) [49] and 38% of PWID had never had an HIV test [50]. Among the sample of PWID, of those who were positive, 62% were unaware of their status [50]. It is estimated that in 2009 testing rates in high risk groups are still low, with 27% of MSM, 52% of CSWs and 47% of PWID having had a test in the last 12 months and knew the result [1].

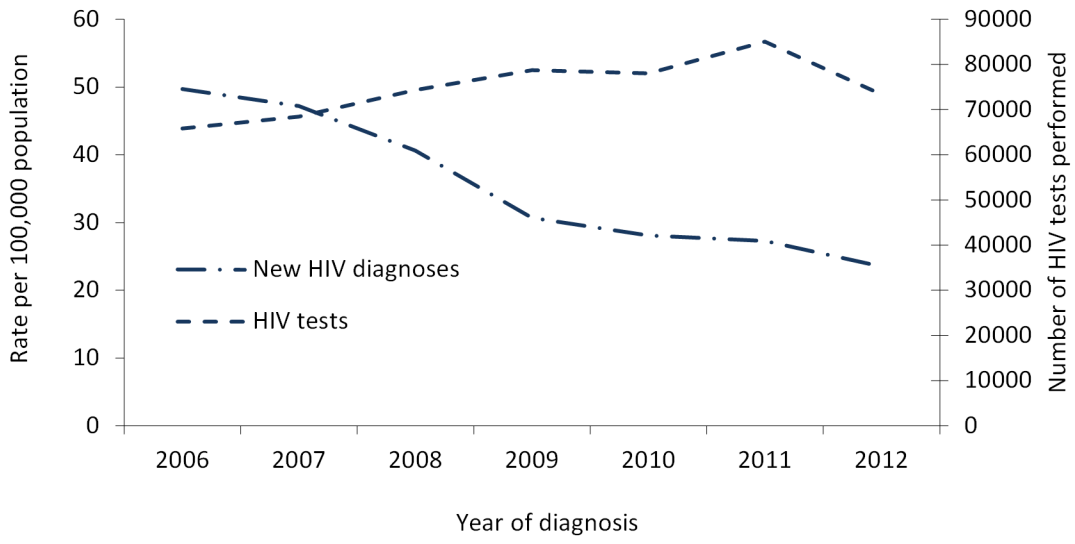


Figure 1.8: Rate of new HIV diagnoses and number of HIV tests performed, Estonia: 2001-2010. Source: European Centre for Disease Prevention and Control [15].

Changes in the leading route of infection were instrumental to the rise of new diagnoses, changing from sexual contact (both sex between men and women and sex between men) to injecting drug use. In 2001 the number of new HIV diagnoses had peaked at 1,474, of which PWID accounted for 90% of diagnoses [51]. In more recent years the proportion attributable to PWID has decreased (48% in 2009) [51] (figure 1.9), a positive outcome of the needle exchange projects which were piloted in 2007 increasing from 13 sites to 36 in 2009 [51]. However prevalence is high with studies conducted between 2002 and 2009 reporting the HIV prevalence among PWID ranging from 45-56% [50, 52, 53], with estimated incidence reported as 20.9 per 100 person years in 2005 and 2007 and 9.0/100 pyrs in 2009 [53]. In more recent years diagnoses among heterosexuals have increased and in 2010 the number of diagnoses attributable to heterosexual contact crossed that of PWID [10, 51]. Information on MSM in Estonia is limited; however the prevalence among a sample of 79 MSM in 2008 was 2.5% [48].

The rapid increase in HIV infections in 2000 initially occurred in the North Eastern region of Ida-Virumaa, followed by the capital city Tallinn [51, 54], with these regions still account for the majority of new diagnoses representing 45% and 44% respectively in 2011 (figure 1.10) [55].

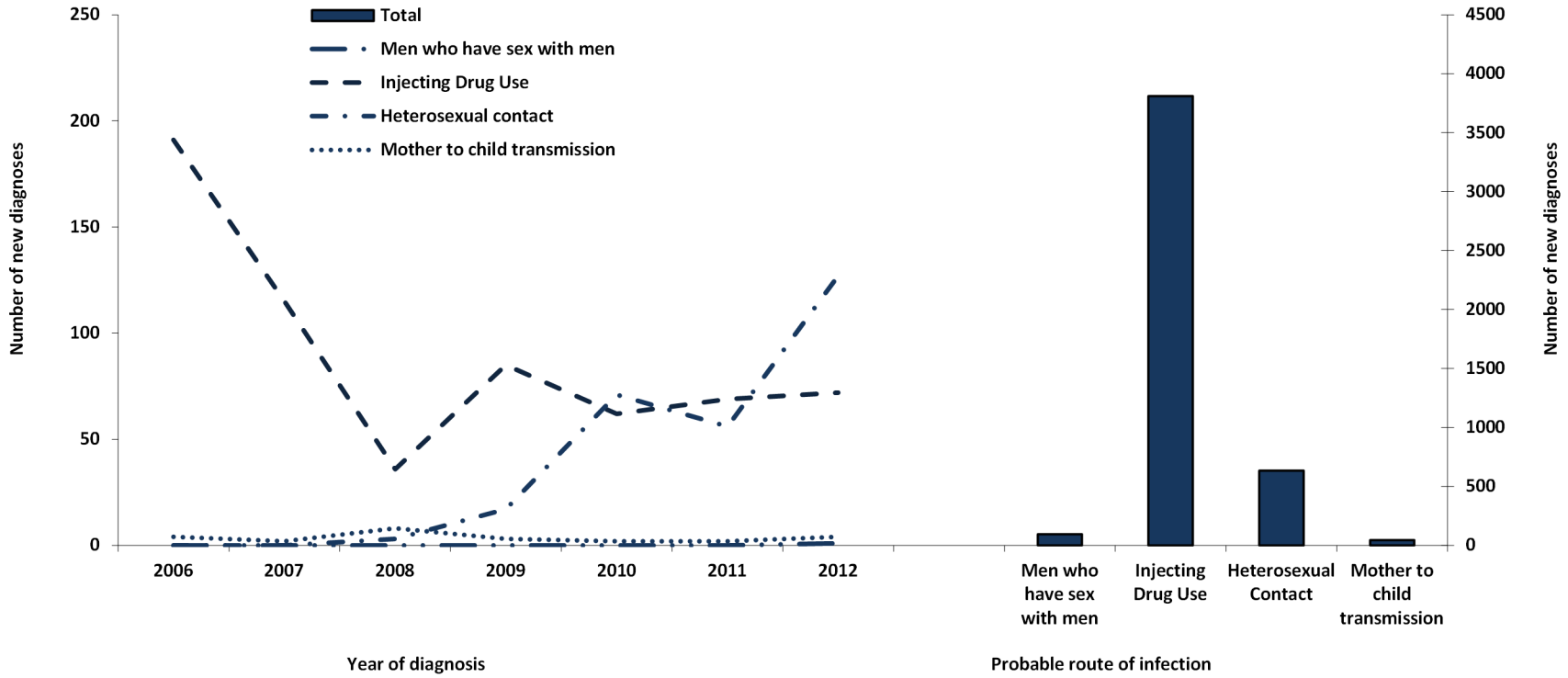


Figure 1.9: Number of new diagnoses by probable route of exposure; line graph represents the number of new diagnoses by probable route of exposure and year of diagnosis; Bar graph represents the number of cumulative diagnoses by probable route of exposure: 1988-2010. Source: European Centre for Disease Prevention and Control [15].

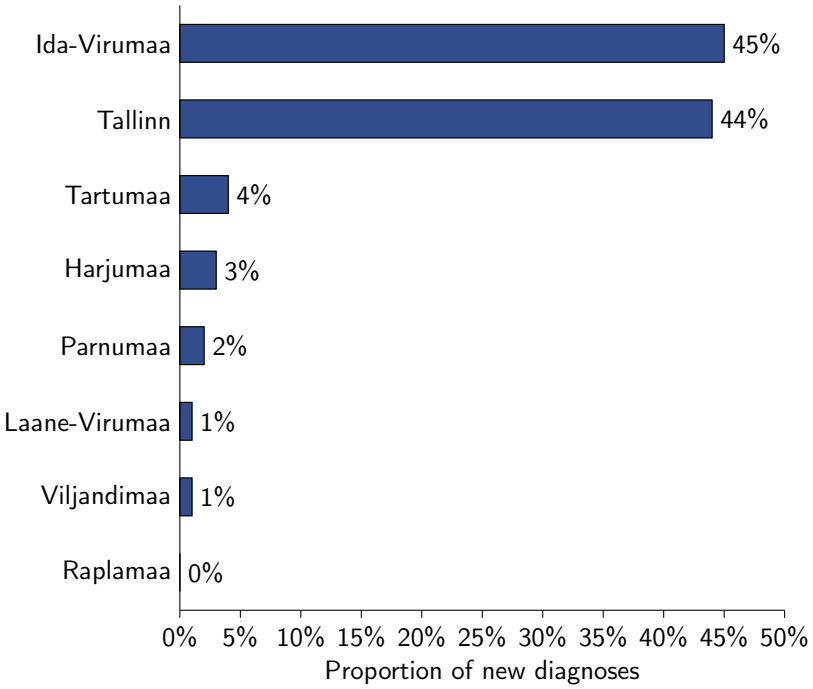


Figure 1.10: Regional distribution of new HIV diagnoses in Estonia: 2011.
Source: Terviseamet Health Board, 2010 [55].

1.6 Focus of Thesis

The aim of this thesis is to identify and characterise new HIV diagnoses and recent HIV infections in three counties within Eastern and Central Europe; Estonia, Poland and Ukraine. This is addressed by introducing a relevant methodology for identifying recent infections and enhance current surveillance methods to:

1. explore the distribution of persons newly diagnosed within in each country,
2. estimate robust incidence rates for each country,
3. validate methodologies for differentiating recent from long standing infections,
4. ascertain risk factors associated with onward transmission, and
5. assess the distribution of patients identified as recently infected.

Chapter one describes the distribution of HIV in Europe, and specifically in Estonia, Poland and Ukraine. **Chapter two** outlines the different methods which have been used to estimate HIV incidence and their limitations. **Chapter three** defines further the methods and definitions behind the recent infection testing algorithm, **Chapter four** explores the statistical methods used to estimate incidence and their limitations. **Chapter five** describes the datasets used in the thesis, methods for data collection and modifications applied. **Chapter six** describes the characteristics of persons newly diagnosed during the study period for each country and derives a number of components needed for estimating incidence. **Chapter seven** describes the characteristics of persons recently infected during the study period for each country, incidence estimates for each country, the circulating HIV Subtype and the distribution of transmitted drug resistance among those recently infected. **Chapter eight** discusses findings and methodologies with suggested recommendations.

Chapter 2

Methological Approaches to Estimating Incidence

Using published literature I outline methodologies for estimating incidence, identifying studies which have used these methodologies and present incidence estimates for the most recent five years.

2.1 Introduction

Estimating HIV incidence is an essential public health monitoring tool, characterising those at risk of HIV infection within a defined population. Incidence estimates enable development and evaluation of appropriate healthcare interventions, resource planning and allocation, monitoring of current trends in the epidemic and reducing onward transmission. Case-reporting of HIV, already established in many European countries, enables estimates of the prevalence of the disease to be made, establishing its magnitude within the population. However, this prevalence rate does not necessarily reflect incidence patterns and cannot distinguish whether the increase is due to onward transmission or a result of increased uptake of testing. This issue arises from the time lag between when an individual is infected and subsequently diagnosed. The long latent period for HIV means that an HIV-infected individual can live for many years without knowing their HIV status. This is evident in the high proportion of individuals in Europe who are diagnosed after a stage at which treatment should already have begun [10], by either being newly diagnosed with a CD4 cell count less than 350 cell/mm^3 or with an AIDS defining illness. This major limitation results in the prevalent snapshot failing to ascertain a true picture of the current epidemiological situation. As a result, numerous methodologies have been implemented and evaluated in an attempt to make robust incidence estimates to understand HIV transmission patterns.

The main objectives of this literature review is to identify methodologies which have been used for estimating incidence, and review studies which have applied and evaluated these methods. Through reviewing papers, abstracts and national reports I have given a detailed account of the most frequently used methodologies for estimating incidence and discuss the performance and limitations which arise from each.

2.2 Literature Review Search Methodologies

I conducted a literature review using the PubMed electronic database, manual searches of bibliographies and searching for grey literature through Google scholar, and abstracts from three major conferences (International AIDS Society (IAS), the Conference on Retroviral and Opportunistic Infections (CROI) and the Joint Statistical meeting (JSM)). Key words and search criteria were used throughout the searches to ensure consistency and different combinations were investigated to identify the most comprehensive search. Search criteria used (table 2.1) were medical subject headings or Medical Subject Headings (MeSH) terms which are used for indexing articles and words specifically identified in the Title and Abstract (TIAB). Two separate searches were conducted in the PubMed electronic database, search a). HIV [MeSH] and Incidence [MeSH] AND (recent [TIAB] OR incidence [TIAB]) and search b). HIV incidence [All fields].

Key Word	**Search Criteria**	**Results**
1. HIV	MeSH	71,404
2. Incidence	MeSH	145,619
3. Recent	TIAB	565,859
4. Incidence	TIAB	438,559
5. RITA	TIAB	634
6. STARHS	TIAB	39
7. Detuned Assay	TIAB	13
8. HIV Incidence	TIAB	1024
9. Incidence Estimation	TIAB	57
10. Incidence Assay	TIAB	9
11. BED	TIAB	56,491
12. 1 AND 2		1086
13. 3 to 11 (OR)		1,031,222
14. 3 OR 4 OR 5 OR 6 OR 11		1,031,222
15. 1 AND 2 AND 14		676
16. 1 AND 2 AND 3 OR 4		689
17. HIV Incidence	All Fields	890

Table 2.1: Search criteria used for identifying literature through the PubMed electonic database.

2.2.1 Pubmed Electonic Database

Two separate searches were conducted in the PubMed electronic database, search a) "HIV" [MeSH] AND "Incidence" [MeSH] AND ("Recent" [TIAB] OR "Incidence" [TIAB]) and search b) "HIV incidence" [All fields]. Although all the key words previously identified were included in the initial development of search a, those key words that did not offer any further literature were dropped; the key word manipulation can be seen in the table. Search b was conducted to incorporate any possible literature which might have been missed through the search a.

The initial literature search identified 689 publications for search a and 890 for search b. Titles and abstracts for these publications were appraised for inclusion, only English language papers were reviewed. Final inclusion included 102 publications for search a (figure 2.1) and 105 for search b (figure 2.2).

2.2.2 Manual Bibliography Search

A manual bibliography search was conducted on all literature identified through the PubMed electronic database search to identify any key publications which were not captured through the initial search. Sixty publications were identified and included in the literature review.

2.2.3 Second Search

A second literature search was conducted in August 2013 using both methods indicated in section 2.2.1 and section 2.2.2. 52 additional publications were identified and included in the literature review.

2.2.4 Conference Proceedings

A manual search was conducted on all abstracts accepted for either an oral or poster presentation at the three conferences indicated previously (IAS, CROI and JSM) to identify any key work which has not yet been published. Forty-six abstracts were identified and included in the literature review.

2.2.5 Grey Literature

Google Scholar and the search engine Google were used to identify grey literature. The majority of these documents were produced by multinational organisations including the World Health Organisation (WHO) and Joint United Nations Programme on HIV/AIDS (UNAIDS) and the European Union agency the ECDC previously EuroHIV. Three reports were identified and included in the literature review.

Figure 2.1: Review process to establish relevant literature on methods for estimating incidence and identifying incidence estiamtes using search A.

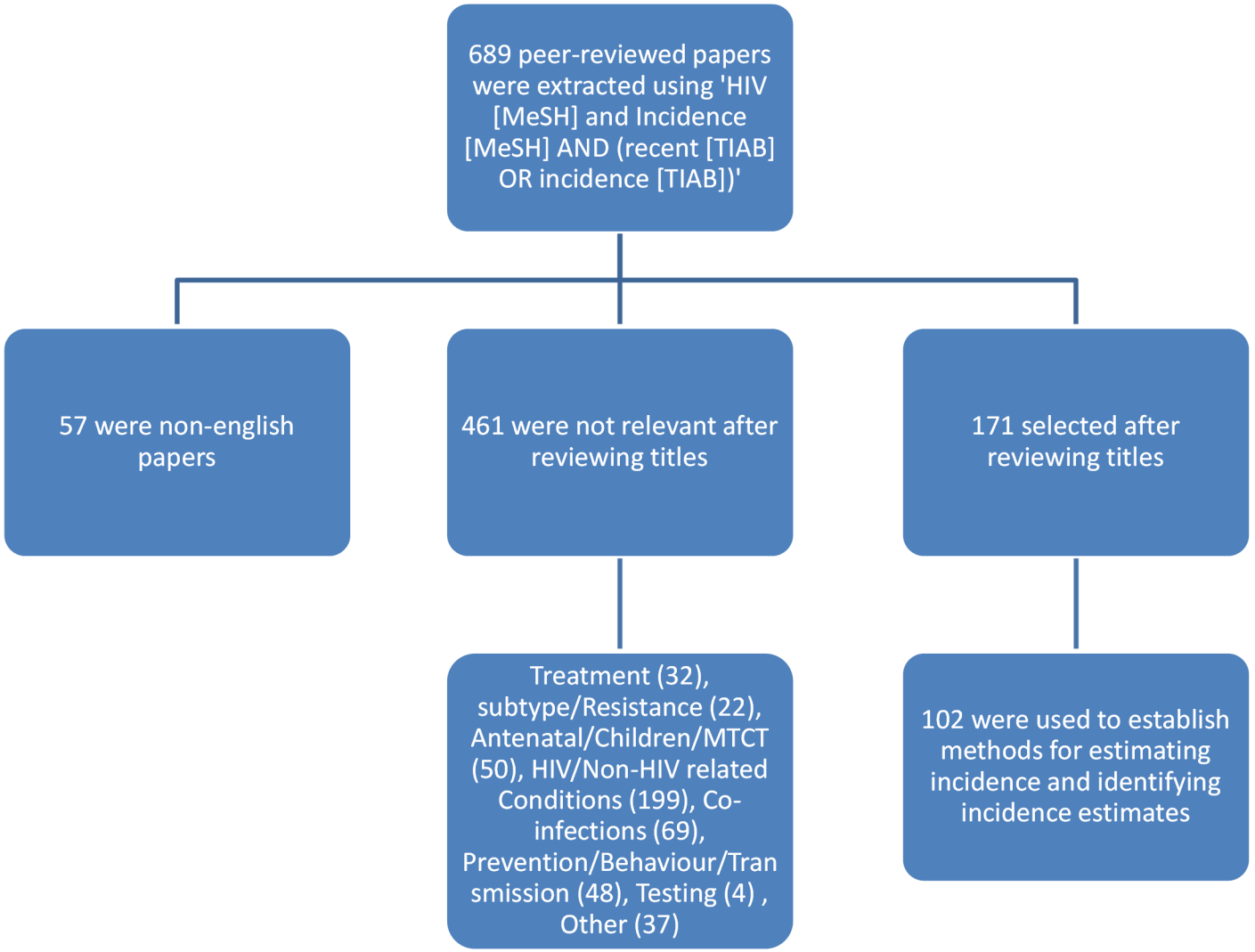
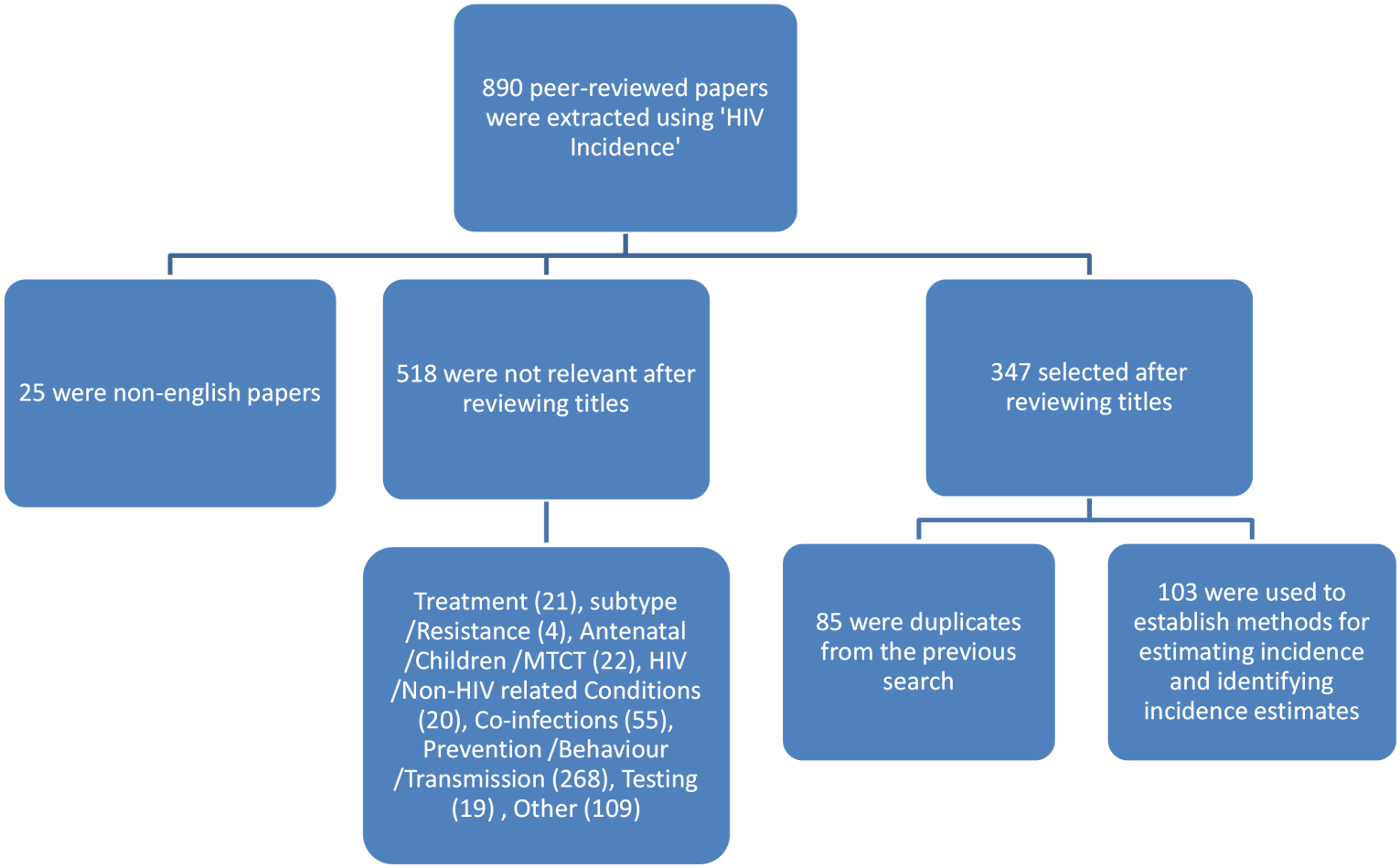


Figure 2.2: Review process to establish relevant literature on methods for estimating incidence and identifying incidence estiamtes using search B.



2.3 Methods for Estimating Incidence

I identified three main methods through reviewing the literature: longitudinal studies, studies using serological testing, and studies using sero-positive data. Overall, I identified 328 publications which used one or more of the three methodologies, or discussed the methodologies, with the majority using serological methods and, in particular, RITA. Within this section I will discuss the different methods used to derive incidence estimates, and discuss the limitations of each method and interpretation of derived results.

2.3.1 Longitudinal Studies

Longitudinal studies are a direct method for measuring incidence, whereby persons who are initially without the disease of interest are followed-up over time. New infections are identified during this time period through regular follow-up allowing time of infection to be estimated as the midpoint between the last negative date and first positive date. Incidence is, therefore, the number of new infections per person-years at risk[N/PY].

Ideally a cohort would follow the population prospectively, starting with a sero-negative population, and through regular follow-up or the patient returning for at least one follow-up visit, new infections can be identified. This method allows incidence to be directly measured and multiple exposures to be considered. However, this method is time consuming, expensive and, depending on the level of incidence within the population, could require several years in duration to collate meaningful estimates. A further issue is the representativeness of the population; cohort studies are susceptible to loss to follow-up with those remaining under follow-up having different experiences and, therefore, HIV incidence rates, from those lost to follow-up. A study among MSM indicated that compared to MSM who remained in the study, those who were lost to follow up were significantly more likely to be risky and therefore have higher incidence [56]. There may also be changes in behaviour among the participants as information on risk reduction and prevention measures become available and are taken up by them.

An alternative method is the retrospective cohort which uses information on all persons

presenting for a test at a particular site regardless of their HIV status. For these persons, the testing history either held at the testing site or self-reported by the individual is used to estimate HIV incidence. However, with a retrospective cohort the appropriate denominator needed for estimating incidence is not known. In addition, repeat testers could lead to an over-estimation of incidence, repeat testing because of high risk behaviour, or an under-estimation where engaging with health services results in lower risks.

2.3.2 Sero-positive data

Indirect methods of estimating incidence are based on HIV diagnoses: by testing of populations through serial sero-surveys, and by use of mathematical models. Primarily based on data already collected, these methods are much less costly.

Probably the most recognised use of sero-positive data is the Estimation and Projection Package (EPP) used by UNAIDS and the WHO to calculate country specific short-term estimates including incidence [57–65]. Estimates are generated by fitting an epidemic model to the observed prevalence data, with additional assumptions on survival, sex ratio, effects of HIV on fertility, MTCT rates, age patterns and the effects and coverage of treatment [58]. Four parameters are used to estimate the best fit; 1. the rate of growth of the epidemic; 2. the fraction of the population at risk of infection at the start of the epidemic; 3. the starting year of the epidemic, and 4. a parameter that modulates recruitment to the at-risk population in response to mortality-driven declines in the population overtime [58]. This model has been reviewed every two years since its introduction in 2003, with modifications allowing for movement between populations [60], fluctuations in prevalence [61]. More recently, modifications allowing for incidence rates to vary over time with long running epidemics [64, 65] and the use of population based CD4 cell counts, estimates of MTCT and survival in children [66].

Alternative methods for estimating incidence include the use of prevalence data, through consecutive surveys or fixed populations [67–71]. Incidence estimates can be made through linking two or more cross-sectional surveys; determining how many of those participating more than once had seroconverted over time, using changes in sero-prevalence in a fixed population to reflect new infections or by dividing the difference in sero-prevalence between two surveys by

the time between them. Prevalence in younger age groups, particularly among pregnant 15-24 year olds [72, 73], provides an estimate of incidence as young age groups have had less time to acquire an HIV infection sexually and, therefore, are likely to represent incident infections. If the incidence is stable over time in the population of interest, the linear increase of prevalence should reflect HIV incidence [67, 74, 75]. Finally, short-term incidence has been estimated using mode of transmission (MOT) model, estimating the number of new infections occurring among particular risk groups, using the population size of each group, current prevalence, and risk of exposure [76–80].

The Back-calculation method uses temporal data on a disease end point to reconstruct trends in the number of new infections underlying the observed end point time series. This method has been used to estimate past trends in HIV incidence on the basis of temporal data on AIDS diagnosis. The estimated HIV incidence can then been used to provide projections of AIDS incidence [81–93]. In its simplistic form the back-calculation method relies on knowledge of the incubation period between HIV infection and AIDS diagnosis and the number of observed AIDS diagnoses over time. A number of developments to the model have addressed changes in the incubation period with the introduction of treatment [92, 93], test-seeking behaviours [88] , differences in populations and incubation periods such as age [89, 90, 94, 95]. In particular, the changes in the distribution of the incubation period following the introduction of Antiretrovirals (ARVs) and the diminished relevance of the AIDS end point have impacted on the ability to use this method and its usefulness, respectively. Amongst the various developments, a recent formulation of the back-calculation uses HIV diagnosis as disease end point and exploits information on CD4 counts around diagnosis to estimate past HIV incidence. Here HIV progression through CD4 stages and testing uptake from the different disease stages are simultaneously modelled [96–103].

Further mathematical models fit age- and time-specific prevalence data using the maximum likelihood method [70, 104–110]. This method extends the use of prevalence in younger age groups to estimate incidence. A number of adjustments have meant numerous modifications to the maximum likelihood model including influence of calendar time, changes in incubation time overall and between risk groups, mortality and morbidity [111] and, in particular, the effect of the "relative inclusion rate" among the infected and uninfected [104, 106, 107, 109, 110].

Alternatively, a deterministic transmission dynamic model can be used, incorporating data on HIV prevalence, demographic and behavioural data [112] or a Stochastic Simulation Model [113].

2.3.3 Serological Testing

Laboratory-based methods to differentiate recent from long-standing infections using the maturing antibody response to the HIV infection have been developed since the early 1990s. Unlike longitudinal studies which require significant follow-up to identify recent infections, this method uses a single sample of diagnostic serum taken at a single point in time. This methodology is collectively known as RITA, previously the Serological Testing Algorithms for HIV Seroconversion (STARHS), and refers to the laboratory assay and additional clinical information required to estimate incidence. Each serological test uses key components of the antibody response to the virus following infection to identify whether an individual has been recently infected, including antibody concentration, response, reaction or proportion, isotype and avidity (figure 2.3).

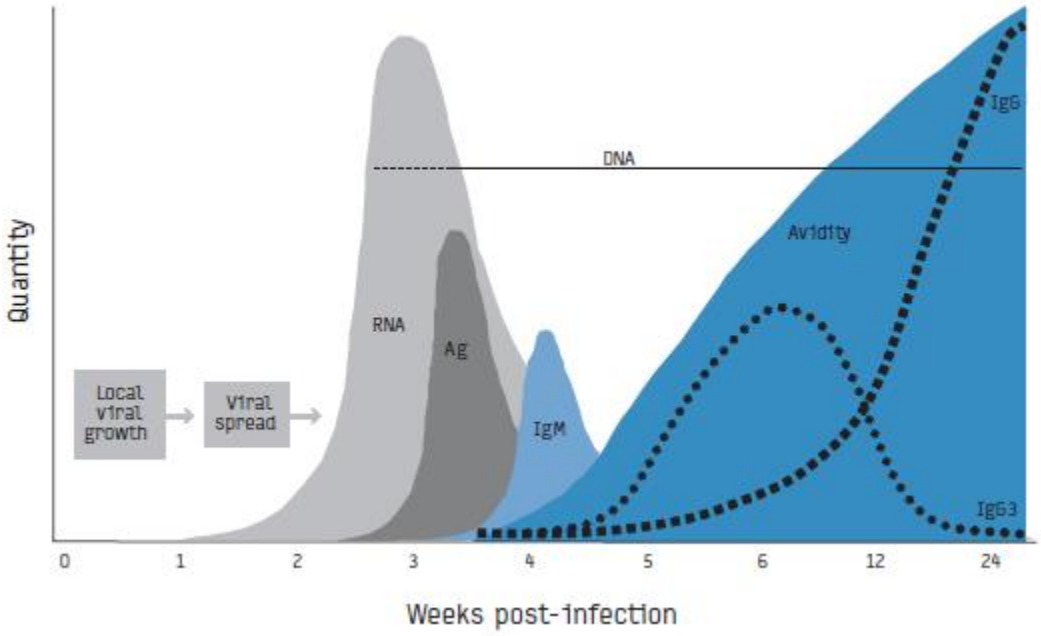


Figure 2.3: Evolution of HIV after initial infection.

Source: Murphy *et al Eurosurveillance* 2008 [4]

There have been eight laboratory methodologies developed, two among those pre-seroconversion and six among the sero-positive population, table 2.2 gives an overview of each method. Based on the biological marker, an optical threshold is derived from the quantified result (Optical Density (OD)) of the anti-HIV immune response measured by the assay defining the point an individual stops being recent (i.e. has an OD below the threshold) and becomes classified as an established infection (i.e. has an OD above the threshold). The time an individual stays below this threshold is referred to as the "Mean Duration of Recent Infection (MDRI)" "window period of recency", "duration of recency", "recency period" or "seroconversion window". This period differs between individuals and between the serological tests. Serially testing samples from individuals with known dates of seroconversion, the OD and corresponding sample date (HIV infection duration) for each person is plotted to establish the optimal threshold which will best define a recent infection. This is achieved through increasing the OD by increments of time to maximise sensitivity and specificity, estimating the mean and median OD by defined time, reaction/response measured by time, linear or non-linear mixed models, non-parametric survival analysis or using the proportion of recent infections among seroconverters. The incidence estimates are then derived from a mathematical formula, details are given in Chapter 4.

RTIA Assay	Overview of methdology
p24 antigen [114, 115]	Identifying the p24 antigen when negative or intermediate for HIV antibodies
HIV RNA [116]	Presence of HIV RNA in the absence of HIV antibodies
Sensitive/Less Sensitive EIA (detuned) [117]	Uses one sensitive and one less sensitive assay to identify rising antibody levels
IgG BED-CEIA [118]	Measures the proportion of HIV-specific IgG antibodies
Avidity [119]	Uses the avidity index (bond between antibodies and antigen)
IDE-V3 EIA [120]	Antibody concentration for identified antigens
IgG3 Isotype [121]	Antibody response to specified antigens
INNO-LIA HIV I/II score [122]	Antibody reaction to specified antigens

Table 2.2: Methodology overview of the RITA assays used to distinguish recent from longstanding infections and their limitations.

There are 3 main frameworks used for estimating incidence: a cross-sectional sample, sentinel surveillance, and through routine testing sites. Cross-sectional studies collect data on a study population, representative of the whole population at a given point in time. Persons are included using a sampling strategy or through convenience sampling based on accessibility using sexual health clinics, bars or community groups. Sentinel surveillance allows detailed information to be collected from a limited number of sites, which could be a population of particular interest or a representative sample of the overall population. For both methods information is available on both the sero-negative and positive population allowing HIV incidence to be estimated using the approach described in Section 4.2.

RITA testing can also be introduced into already established surveillance systems. However, for the majority of studies using surveillance systems data and samples are only available for positive persons, requiring a different approach to estimating incidence (section 4.3).

Although RITA addresses the limitations surrounding longitudinal studies by removing the need for repeat testing, there are still a number of limitations that need to be considered. A fraction of long-standing infections could be classified as recent due to factors impacting a person's immune response. These factors include AIDS or a low CD4 cell count, a low or undetectable viral load or being on treatment at the time of the test [123, 124]. A false recent result could also occur if a person's antibody response remains under the threshold for a prolonged period of time [124, 125]. Further factors found to impact test characteristics are pregnancy, co-infections and HIV sub-type [123, 126–130]. Consideration must also be given to the representativeness and size of the population being tested. These issues will be discussed further in chapter 3.

For the majority of studies where an assay was used but incidence estimates were not derived, the results were presented as the proportion identified as recent and were more likely to have been conducted using only those identified as HIV positive. Alongside the introduction of new methods for detecting a recent infection there has also been a shift from using a cross-sectional framework for collecting data to introducing RITA testing to an already established national surveillance system within the country.

2.3.4 Ambiguous Nucleotides

Also being investigated is the idea that genetic diversity of the virus sequence could be used to define the age of infection. Although this method has not currently been used to estimate incidence specifically, it has been considered as a method to differentiate recent from long-standing infections. Much like many of the biological markers used by the serological test (see section 2.3.3 and Chapter 3) such as the avidity index, genetic diversity, the proportion of ambiguous bases in the HIV-1 pol sequence, increases from a single virus in early infection, suggesting inference of a person's age of HIV infection.

Four studies [131–134] have investigated the use of genetic diversity to differentiate recent from long-standing infections, suggesting that a cut-off of 0.45-0.50% could distinguish an infection ≥ 1 year from chronic (>1 year). However, although there appears to be a correlation between age and genetic diversity, estimated at 0.2% per year [134], all studies found the specificity to be around 70%, with Kouyos *et al* suggesting that genetic diversity may be more helpful at accurately discriminating against chronic infections rather than recent [134].

As a simple and inexpensive method, particularly if transmitted drug resistance is already being assessed this should be considered as a complementary approach to the serological test (RITA), minimising misclassification of long-standing infections as recent.

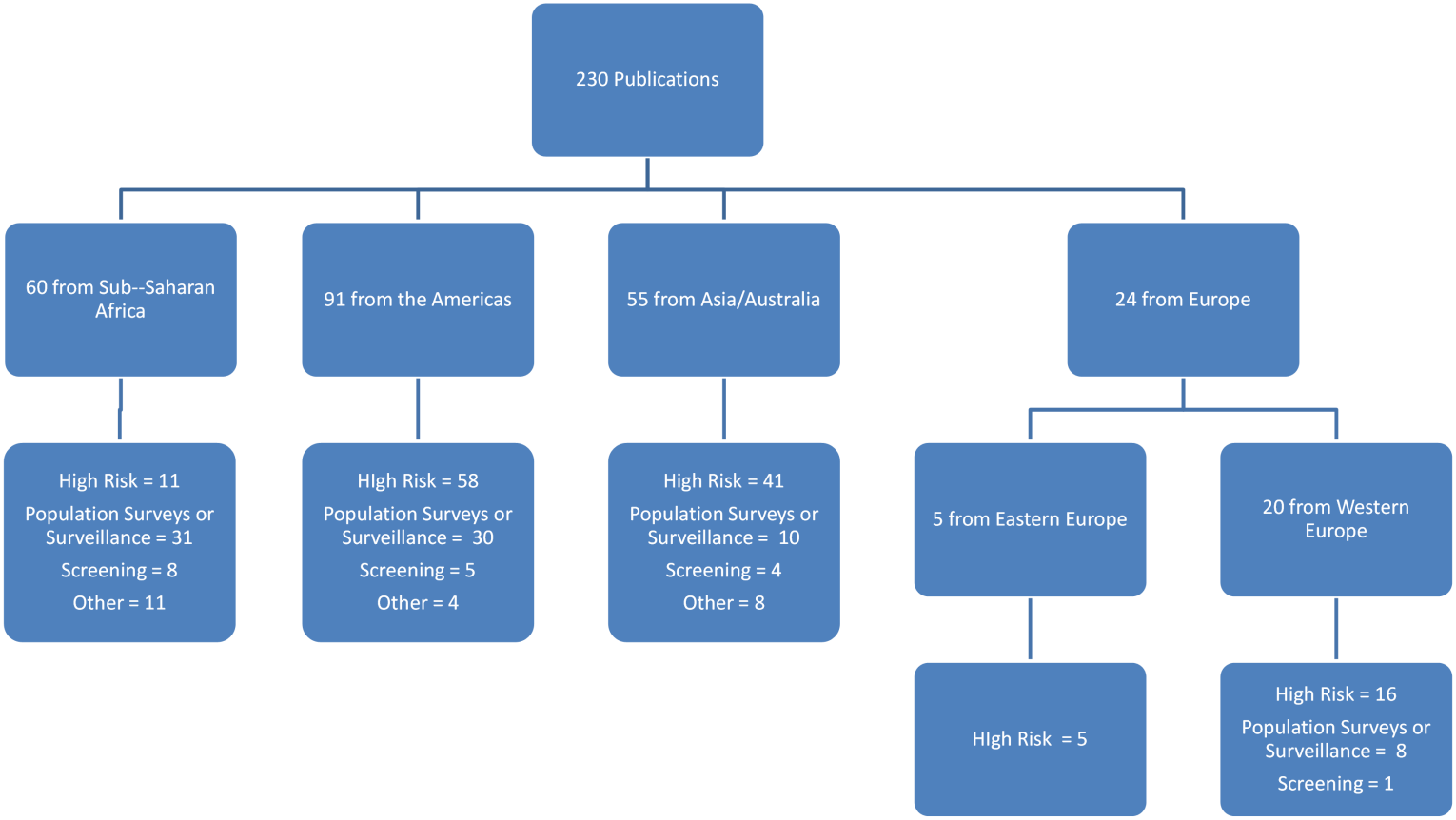
2.4 Overview of Published Incidence Estimates

Using the methods indicated in section 2.2, I identified studies which have estimated incidence using one or more of the three methods discussed. Publications which included incidence estimates were collated and grouped by study region. All incidence estimates were present by 100 pearson years (pyrs) at risk (or of follow-up). Methods were grouped into cohort, RITA and other and populations of focus were grouped into high risk, testing population and other; the distribution of these groups are in appendix A. Studies which did not present incidence estimates were more likely to provide the proportion of persons recently infected and these studies are discussed separately.

2.4.1 Incidence estimates per 100 person-years at risk.

In total I identified 230 studies published over a 20-year period (1994-2013), producing 767 incidence estimates for the years 1984-2011. The distribution of these studies are shown in figure 2.4, with 91 studies within Americas, 55 and 60 in Asia/Australasia and Africa respectively and 24 in Europe (20 in Western Europe and 5 in Eastern Europe). I grouped the study populations into 4 categories, high risk, Population Surveys or Surveillance, Screening and other, with 131, 80, 18 and 22 studies in each group, respectively. Sixteen studies reported estimates for more than one population category and 29 used multiple methods to estimate incidence. Predominately these studies used the cohort method (143) or serological test (105); only a small number used mathematical models. Within these methodological groups, 118 cohorts were prospective [56, 68, 105, 114, 125, 135–245] and 30 retrospective [68, 221, 246–272]. For the serological test the breakdown is as follows: Four studies used p24 antigen to identify recent infections [68, 114, 190, 241], 3 used HIV RNA [190, 231, 273], 43 used the concentration of HIV antibodies (LS-EIA) [162, 232, 252, 256, 259, 274–311], 58 the proportion of HIV specific IgG antibodies (BED-CEIA) [80,125,154,157,159,164,175,178,201,202,224,231,238,240,252,254,263, 273,284,299,307,309,312–346], 11 Avidity index [178,231,240,251,273,284,290,343,344,347,348] and 3 used antibody response to antigens [349–351]. Details of these serological tests can be found in chapter 3.

Figure 2.4: Flow diagram of publications by region and population tested.



Methods differed by region with estimates for Africa more likely to be from a cohort study than RITA (40 vs.17) whereas for Americas and Asia/Australasia there was little difference between the two groups. Differences by country were also seen by study population. African studies were more likely to be from population-based surveys compared to all other regions where the focus was on high risk populations. These populations were representative of the distribution of those at risk, with Americas and Western Europe having a higher number of studies among MSM, Asian CSWs and Eastern Europe PWID.

Focusing on incidence estimates for the most recent five years (2007-2011), where date was reported, 42 studies were included (table 2.5). Asia/Australasia had 13 and Americas had 14 studies each [56, 80, 156, 157, 159, 159, 161, 169, 171, 185, 194, 207, 208, 213, 214, 226, 231, 255, 270, 301, 317, 318, 334, 336, 346], 11 from sub-Saharan Africa [141,182,183,191,195,196,218,219,230,344,352] and 8 in Europe [113,223,227,254,258,348–350]. The majority of studies were among high risk populations (32), followed by Population Surveys or Surveillance (14), Screening (3) and used the cohort method (31), RITA (15) or other (3).

The highest incidence estimates for Africa were among women in South Africa ranging from 5.5 per 100 pyrs [230][152] to 14.8/100 pyrs [195]. Incidence estimates among FSW in Kenya were 5.6 per 100 pyrs [191] and among discordant couples in Uganda 4.3 per 100 pyrs [182]. The lowest incidence estimates were among Police Officers in Tanzania with 0.84 and through community based clinical trials in Zimbabwe and Tanzania with 0.91 and 0.78, respectively [196,344].

Incidence estimates for women in the USA were, as expected, much lower than in Africa with estimates ranging from 0.13 per 100 pyrs to 2.52 [231]. The highest incidence estimates were among MSM in New York with 5.7 per 100 pyrs [255]. PWID in Vancouver were estimated to have incidence of 2.49 [161] and FSW in Honduras 0.23 [80]. Estimates for 50 US states and the District of Columbia in 2009 were 0.019 falling from 0.023 in 2007 [346].

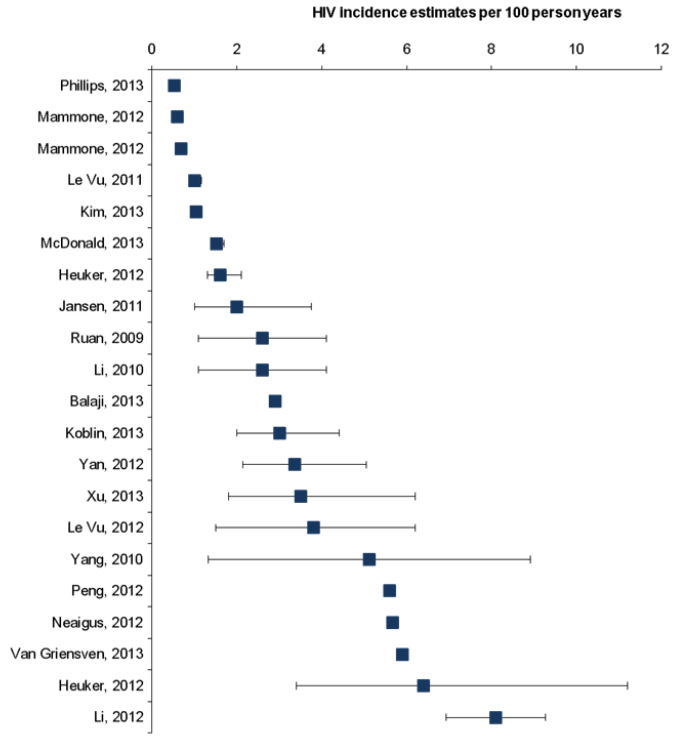
For Asia and Australasia, the majority of incidence estimates were calculated among the three main risk groups MSM, PWID and FSW.PWID from Dehong Prefecture in China had incidence estimates as high as 9.7 per 100 pyrs in 2007 falling to 4.3 in 2008 [318]. Estimates for MSM ranged from 8.09 [207] to 2.6 per 100 pyrs in Beijing [185] and among FSW estimates ranged

from 4.4 per 100 pyrs in Yunnan [353] to 0.6 in Dehong Prefecture [318]. Discordant couples had estimates of 7.2 per 100 pyrs in Dehong Prefecture [318] and pregnant women, pre-martial couples and military personnel all had estimates below 0.25 per 100 pyrs [318, 336].

MSM who had post exposure prophylaxis in Amsterdam had the highest estimates in Western Europe with 6.4 per 100 pyrs [227] with the lowest in Italy with 0.601 per 100 pyrs [348]. Overall estimates for PWID were low ranging from 0.043 to 0.577 per 100 pyrs in Italy between 2007 and 2008 [348]. The lowest estimates were among those newly diagnosed in Italy falling from 0.0208 in 2007 to 0.0199 in 2008 [348] and in France 0.017 per 100 pyrs [349]. Estimates for Easter Europe were available for only one study investigating PWID in Russia and during the period between 2005 and 2008 HIV incidence was 25.5 per 10 pyrs [254].

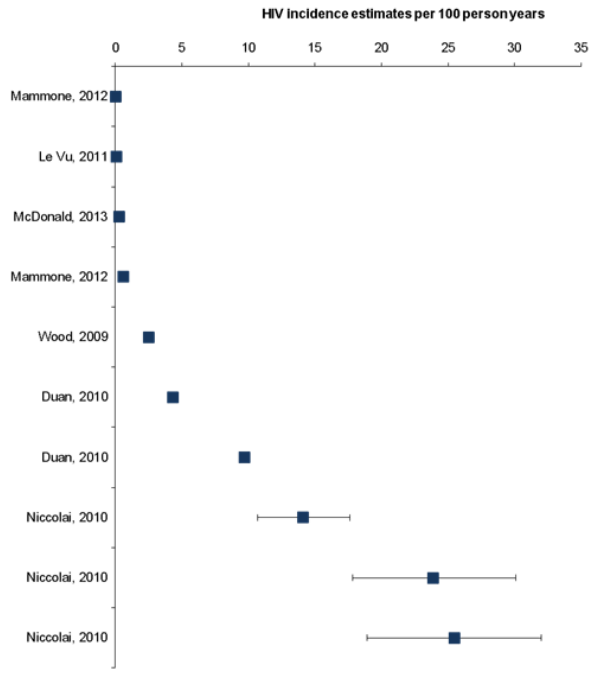
Figure 2.5: Incidence estimates for 2007-2011, by region and population of focus. A. MSM, B. PWID, C.High risk women, D. Persons newly diagnosed and E. Other populations

A.



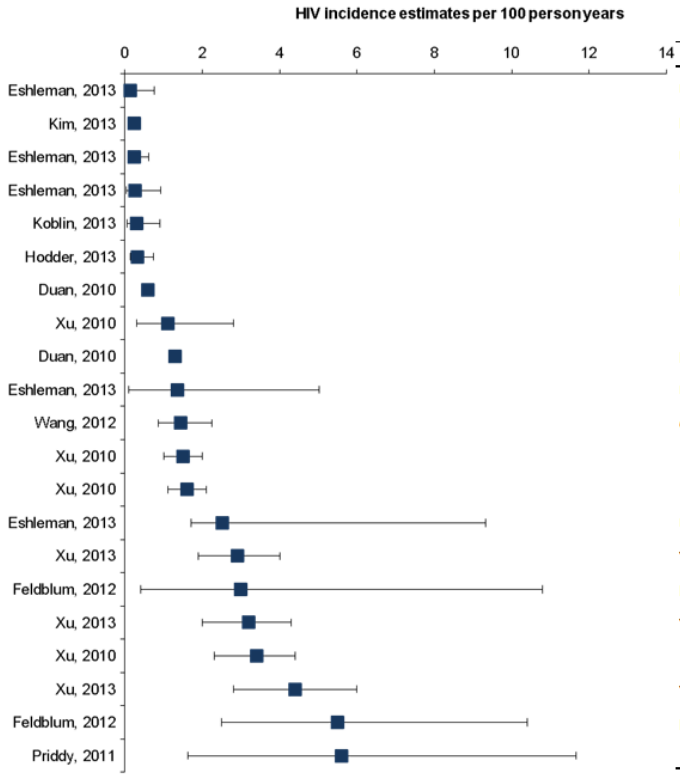
Region-Country	Population	Time Period	Incidence	95% CI	Method
UK	Men who have sex with men	2006-2010	0.53		Stochastic Simulation Model
Italy	Men who have sex with men	2007	0.601		Avidity-previous Negative-RNA/p24
Italy	Men who have sex with men	2008	0.691		Avidity-previous Negative-RNA/p24
France	Men who have sex with men	2008	1.006	0.857-1.155	EIA-RI
Honduras	Men who have sex with men	2007	1.03		MOT
UK - Scotland	Men who have sex with men	1988-2009	1.52	1.38-1.70	Retrospective
Amsterdam	Men who have sex with men	2000-2009	1.6	1.3-2.1	Prospective
Amsterdam	Men who have sex with men	2009	2	1.00-3.75	Prospective
Beijing-China	Men who have sex with men	2006-2007	2.6	1.1-4.1	Prospective
Beijing-China	Men who have sex with men	2006-2007	2.6	1.1-4.1	Prospective
USA	Men who have sex with men	2008	2.9		Retrospective
USA	Black Men who have sex with men	2009-2010	3	2.0-4.4	Prospective
China	Men who have sex with men	2008-2010	3.36	2.13-5.04	Prospective
China	Men who have sex with men	2009-2011	3.5	1.8-6.2	Prospective
Paris-france	Men who have sex with men	2009	3.8	1.5-6.2	EIA-RI
Naujing-China	Men who have sex with men	2008	5.12	1.33-8.91	Prospective
China	Men who have sex with men	2008	5.6		Prospective
New York	Men who have sex with men	2008	5.67		Retrospective
Thailand	Men who have sex with men	2006-2012	5.9		Prospective
Amsterdam	MSM (Post Exposure Prophylaxis)	2000-2009	6.4	3.4-11.2	Prospective
Beijing-China	Men who have sex with men	2009	8.09	6.92-9.26	Prospective

B



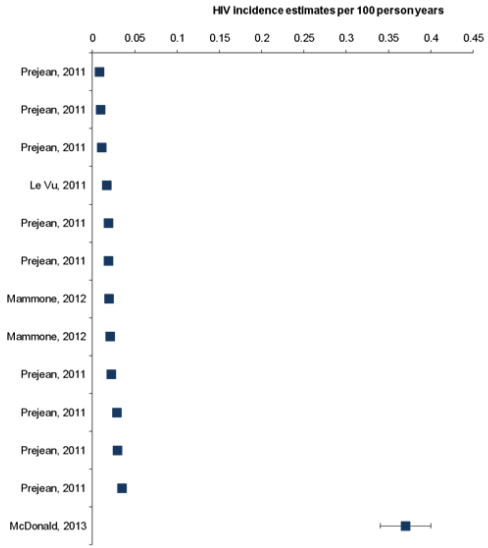
Region-Country	Population	Time Period	Incidence	95% CI	Method
Italy	Injecting Drug Users	2007	0.043		Avidity-previous Negative-RNA/p24
France	Injecting Drug Users	2008	0.086	0-0.0192	EIA-RI
UK - Scotland	Injecting Drug Users	1988-2009	0.27	0.22-0.32	Retrospective
Italy	Injecting Drug Users	2008	0.577		Avidity-previous Negative-RNA/p24
Vancouver	Injecting Drug Users	1996-2007	2.49	2.09-2.88	Prospective
Dehong Prefecture-China	Injecting Drug Users	2008	4.3		BED-CEIA Hargrove
Dehong Prefecture-China	Injecting Drug Users	2007	9.7		BED-CEIA Hargrove
Russia	Injecting Drug Users	2005-2008	14.1	10.7-17.6	Retrospective
Russia	Injecting Drug Users	2005-2008	23.9	17.8-30.1	BED-CEIA Hargrove
Russia	Injecting Drug Users	2005-2008	25.5	18.9-32.0	BED-CEIA McDougal

C



Region-Country	Population	Time Period	Incidence	95% CI	Method
USA	High risk women	2009-2010	0.13	0.006-0.76	MAA (End of Study)
Honduras	Female Sex Worker	2007	0.23		MOT
USA	High risk women	2009-2010	0.24	0.07-0.62	Prospective
USA	High risk women	2009-2010	0.25	0.03-0.93	MAA (Enrolment)
USA	High Risk Women	2009-2010	0.31	0.06-0.91	Prospective
USA	High risk women	2009-2010	0.32	0.14-0.74	Prospective
Dehong Prefecture-China	Female Sex Worker	2007	0.6		BED-CEIA Hargrove
Kaiyuan City-China	Female Sex Worker	2006-2007	1.1	0.3-2.8	Prospective
Dehong Prefecture-China	Female Sex Worker	2008	1.3		BED-CEIA Hargrove
USA	High risk women	2009-2010	1.36	0.091-5.02	RNA (26 day window)
China	Female Sex Worker	2006-2009	1.44	0.87-2.24	Prospective
Kaiyuan City-China	Female Sex Worker	2006-2007	1.5	1.0-2.0	BED-CEIA McDougal
Kaiyuan City-China	Female Sex Worker	2006-2007	1.6	1.1-2.1	BED-CEIA Hargrove
USA	High risk women	2009-2010	2.52	1.7-9.33	RNA (14 day window)
Yunnan, China	Female Sex Worker	2006-2007	2.9	1.9-4.0	BED-CEIA McDougal
Rustenburg-RSA	High risk women	2008-2009	3	0.4-10.8	Prospective
Yunnan, China	Female Sex Worker	2006-2007	3.2	2.0-4.3	BED-CEIA Hargrove
Kaiyuan City-China	Female Sex Worker	2006-2007	3.4	2.3-4.4	BED-CEIA
Yunnan, China	Female Sex Worker	2006-2007	4.4	2.8-6.0	BED-CEIA
Bloemfontein-RSA	High risk women	2009	5.5	2.5-10.4	Prospective
Kenya	Female Sex Worker	2008	5.6	1.62-11.67	Prospective

D



Region-Country	Population	Time Period	Incidence	95% CI	Method
50 US states and the district of columbia	New diagnoses (women)	2009	0.0086	0.007-0.010	BED-CEIA
50 US states and the district of columbia	New diagnoses (women)	2008	0.0095	0.008-0.011	BED-CEIA
50 US states and the district of columbia	New diagnoses (women)	2007	0.0107	0.009-0.012	BED-CEIA
France	New Diagnoses	2008	0.017	0.015-0.019	EIA-RI
50 US states and the district of columbia	New diagnoses	2008	0.019	0.017-0.021	BED-CEIA
50 US states and the district of columbia	New diagnoses	2009	0.019	0.017-0.021	BED-CEIA
Italy	New Diagnoses	2008	0.0199		Avidity-previous Negative-RNA/p24
Italy	New Diagnoses	2007	0.0208		Avidity-previous Negative-RNA/p24
50 US states and the district of columbia	New diagnoses	2007	0.0225	0.02-0.025	BED-CEIA
50 US states and the district of columbia	New diagnoses (Men)	2008	0.029	0.025-0.032	BED-CEIA
50 US states and the district of columbia	New diagnoses (Men)	2009	0.0298	0.026-0.034	BED-CEIA
50 US states and the district of columbia	New diagnoses (Men)	2007	0.0349	0.030-0.039	BED-CEIA
UK - Scotland	New Diagnoses	1988-2009	0.37	0.34-0.40	Retrospective

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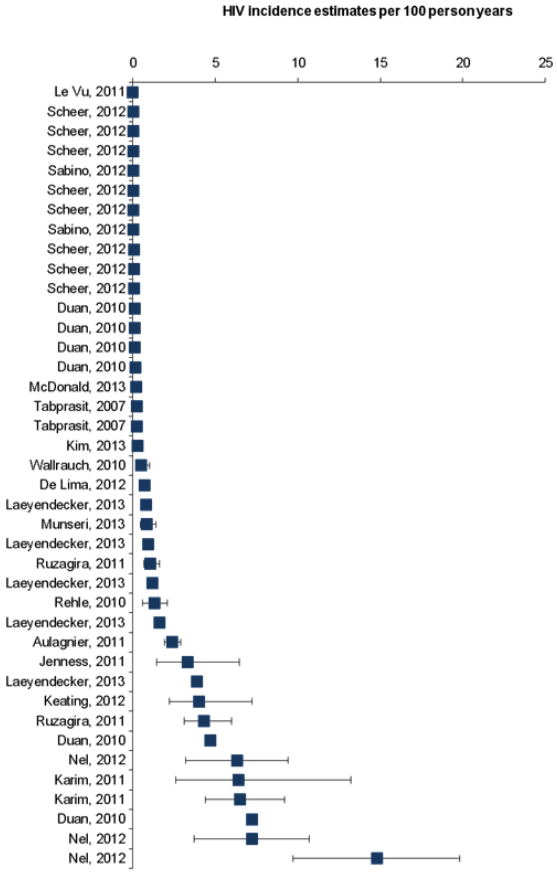


Region-Country	Population	Time Period	Incidence	95% CI	Method
France	Heterosexuals	2008	0.0009	0.0007-0.0010	EIA-RI
California (Excluding Los Angeles and San Francisco)	Adults	2008	0.013	0.009-0.017	BED-CEIA
California (Excluding Los Angeles and San Francisco)	Adults	2009	0.015	0.011-0.020	BED-CEIA
Los Angeles	Adults	2008	0.02	0.016-0.024	BED-CEIA
Brazil	Repeat Blood Donors	2007-2008	0.02	0.017-0.028	Detuned
Los Angeles	Adults	2009	0.023	0.017-0.028	BED-CEIA
Los Angeles	Adults	2007	0.029	0.022-0.036	BED-CEIA
Brazil	First-time Blood Donors	2007-2008	0.04	0.03-0.05	Detuned
san Francisco	Adults	2009	0.052	0.037-0.067	BED-CEIA
san Francisco	Adults	2008	0.065	0.049-0.082	BED-CEIA
san Francisco	Adults	2007	0.07	0.048-0.092	BED-CEIA
Dehong Prefecture-China	Pregnant Women	2008	0.1		BED-CEIA Hargrove
Dehong Prefecture-China	Pregnant Women	2007	0.1		BED-CEIA Hargrove
Dehong Prefecture-China	Pre-marital Couples	2008	0.13		BED-CEIA Hargrove
Dehong Prefecture-China	Pre-marital Couples	2007	0.16		BED-CEIA Hargrove
UK - Scotland	Heterosexual	1988-2009	0.21	0.18-0.24	Retrospective
Thailand	Military	2009	0.25	0.17-0.32	BED-CEIA
Thailand	Military	2006-2008	0.26	0.19-0.36	BED-CEIA
Honduras	Garifuna	2007	0.3		MOT
USA	Adults (≥50yrs)	2006-2008	0.5	0.3-1.0	Prospective
Brazil	Voluntary counselling and testing	2006-2009	0.73	0.61-0.86	BED-CEIA
Tanzania	Community Based Clinical Trial	2009-2011	0.78		Multiassay Algorithm
Der es Salaam, Tanzania	Police Officers	2005-2008	0.84	0.468-1.403	Prospective
Zimbabwe	Community Based Clinical Trial	2009-2011	0.91		Multiassay Algorithm
Masaka, Uganda	Community-based HIV Vaccine Cohort	2004-2007	1.04	0.68-1.59	Prospective
Soweto, South Africa	Community Based Clinical Trial	2009-2011	1.18		Multiassay Algorithm
South Africa	National HIV household survey (15-49yrs)	2005-2008	1.3	0.6-2.1	Two Cross-sectional Prevalence Measurements
Africa	Community Based Clinical Trial	2009-2011	1.6		Multiassay Algorithm
Windhock, Nambia	Household Survey	2007-2009	2.4	1.9-2.9	prospective
New York	High Risk Heterosexuals	2007	3.31	1.43-6.47	Detuned
Vulindlela, South Africa	Community Based Clinical Trial	2009-2011	3.9		Multiassay Algorithm
Malawi	Antenatal clinic	2009	4	2.2-7.2	Prospective
Masaka, Uganda	Discordant Couples	2006-2009	4.3	3.1-6.0	Prospective
Dehong Prefecture-China	Discordant Couples	2008	4.7		BED-CEIA Hargrove
KwaZulu-Natal, Edendale	Women (18-35 yrs)	2007-2009	6.3	3.2-9.4	prospective
KwaZulu-Natal	Women - Urban STD clinic	2004-2007	6.4	2.6-13.2	Prospective
KwaZulu-Natal	Women - Rural family planning clinic	2004-2007	6.5	4.4-9.2	Prospective
KwaZulu-Natal, Pinetown	Women (18-35 yrs)	2007-2009	7.2	3.7-10.7	prospective
Dehong Prefecture-China	Discordant Couples	2007	7.2		BED-CEIA Hargrove
KwaZulu-Natal, Ladysmith	Women (18-35 yrs)	2007-2009	14.8	9.7-19.8	prospective

2.4.2 Proportion identified as recent

I also identified studies which presented the proportion of recent infections within a population [354–380]. The majority of these studies were focused on persons newly diagnosed with HIV, following the introduction of testing for recent infections as part of the surveillance of HIV. As this method in many cases only includes those persons newly diagnosed, estimating incidence is much more complicated due to the limited information on the negative populations. The populations recently infected among new diagnoses varied from 11% in the UK to 35% in Australia (figure 2.6).

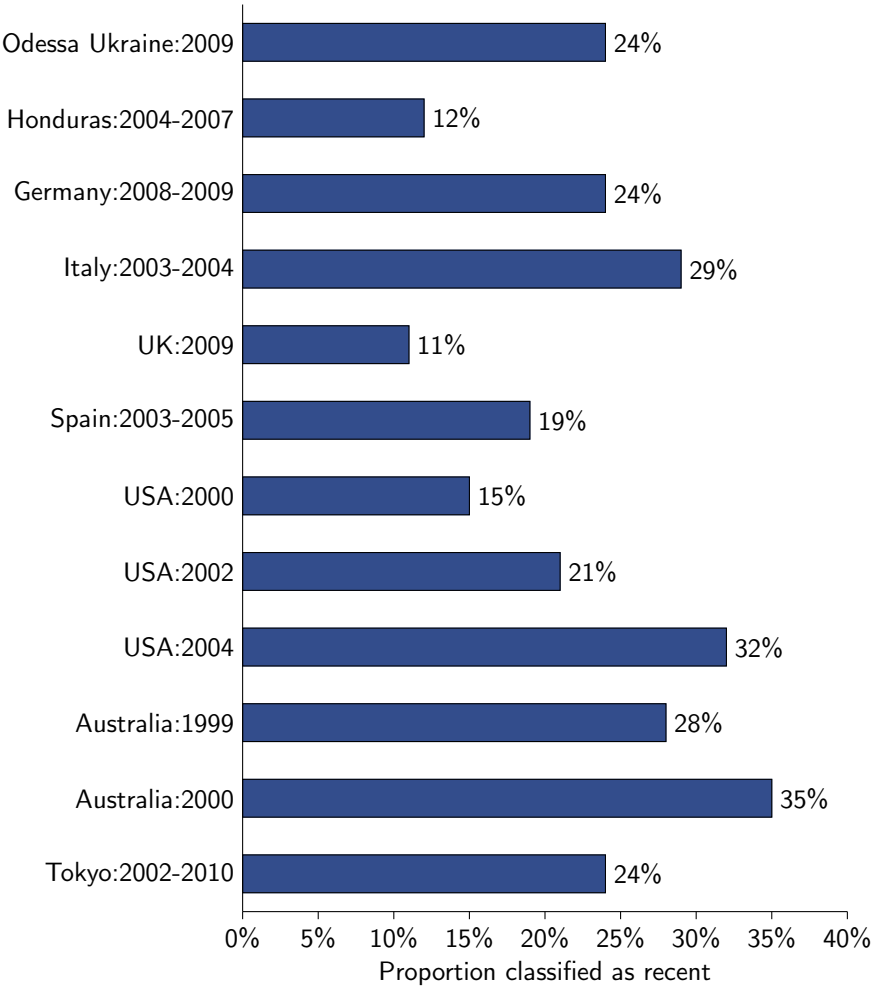


Figure 2.6: Proportion of recent infections among newly diagnosed persons by country and year of diagnosis.

2.5 Summary

Although most countries monitor their epidemics through sentinel or country based surveillance, this method is limited to understanding HIV prevalence and retrospective transmission patterns. To truly understand the dynamics of the current epidemic there is a need for timely and robust incidence estimates.

Through published literature I identified three methodologies which have been used to estimate HIV incidence: following a cohort of sero-negative individuals, sero-positive data collected through routine surveillance and using the maturing response of the virus through a laboratory test. It is important to note that there is no gold standard method for estimating HIV incidence.

Following a cohort of sero-negatives is a direct method for estimating HIV incidence identifying those who become sero-positive. Although direct, this method requires a long period of time for follow-up, especially if the incidence rate is low, and a large amount of expenditure. Where information on testing history is available it is possible to work backward using a retrospective cohort of all individuals testing for HIV at an identified site. The time of infection is estimated as the mid-point between an individual's previous negative and first positive test. Determining the time of incident infection from previous testing history reduces the need for time and money but introduces a number of biases. A person's reason for repeat testing could result in an over-estimation of incidence, testing because of high-risk behaviours or an under-estimation as those who engage with health services maybe at lower risks of HIV.

Alternatively, using sero-positive data already collected is an indirect method to estimate HIV incidence through serial surveys or mathematical models. These methods are relatively inexpensive as they largely depend on data already collected. However, they are vulnerable to changes in the epidemic, such as the introduction of treatment, and are dependent on the assumptions of the mathematical model, which may lead to unreliable estimations.

Finally, the RITA assay which uses key components of the maturing antibody response to the virus to determine whether an individual is recently infected or long-standing, has been a practical alternative to prospective and retrospective cohorts. This method uses a serological

test on a single serum sample taken at a single point in time, requiring no follow-up, and can be introduced to surveillance and laboratory methodologies already in place. Although addressing the limitations which arise through longitudinal and sero-prevalence methods, the RITA assay has its own limitations. Ensuring the correct assay is chosen and that it has been validated for use in the population of interest is extremely important. Furthermore, key components such as the sampling framework, sample size and methods for limiting the possibility of misclassified results need serious consideration which I discuss in Chapter 3.

In additional but not specifically for estimating incidence, is the use of genetic diversity to differentiate recent from longstanding infections. Although there does appear to be a proportional cut-off which indicates recent from longstanding infections, this methodology currently is best used to discriminate those infections >1 year rather than those ≥ 1 year. Where transmitted drug resistance is already being assessed this methodology should be considered particularly in conjunction with the serological test.

Incidence estimates per 100 pyrs for the past five years were presented by geographical region and population. Although the distribution of populations investigated reflect the epidemic within each region, making comparisons between estimates is difficult due to the lack of consistency in the methods used to identify populations and statistical methods to estimate incidence. The introduction of a test for recent infection to national HIV surveillance will allow for a wider comparison between risk groups, regions and years. In the meantime, what could be determined were the populations at greater risk of HIV within particular regions with higher incidence estimates more likely to be among populations such as MSM, PWID and CSWs.

The introduction of RITA within HIV testing facilities was the technique used to estimate incidence rates and understand the current transmission dynamics for the three countries identified in this project. Although there is no gold standard of estimating incidence, this technique will enable timely estimates to be made and to develop tailored prevention and intervention efforts, reducing onward transmission in countries where the diagnostic rates are the highest in Europe and to focus resource planning and allocation.

Chapter 3

Serological Tests for Identifying Incident Infections

Within this chapter, through literature identified in the methods section of chapter 2, I will review the methods developed for distinguishing a recent infection using biological markers, the corresponding limitations and the key components needed for estimating incidence.

3.1 Introduction

The initial serological method for differentiating recent from long-standing infections was introduced in 1998 [117], leading to the development of multiple techniques, collectively known as the RITA or theSTARHS. Each serological method uses key components of the antibody response to the virus following infection, illustrated in figure 2.3, such as antibody concentration, proportion, isotype and avidity.

Following initial infection, HIV RNA and a protein component of the virus, p24 antigen, are usually present for a short period of time before HIV antibodies are detected, after which time only HIV RNA remains detectable. Antibody reactions follow with an initial peak and fall in immunoglobulin M (IgM) followed by a gradual increase in immunoglobulin G (IgG). The avidity index, the strength of the bond between antibody and antigen, also increases following the development of HIV antibodies [4].

These initial components after infection may be broken down into six so called Fiebig stages [381]: stage I RNA positive, stage II RNA and p24 antigen positive, stage III RNA, p24 antigen and HIV IgM-sensitive EIA reactive, stage IV same as stage III but indeterminate Western blot, stage V same as stage IV but Western blot reactive (excluding p31), and stage VI same as stage V but full western blot reactive. Stages I-IV are estimated to have an average time-frame of 3-5 days each, stage V 69.5 days and stage VI is open ended. As a result, the identification of particular markers or early levels of immune response can indicate a recent infection.

3.2 Serological Tests for Identifying Incident Infections

I identified published literature on eight different methodologies developed for detection of recent infection, which include both purposely developed assays and commercially available diagnostic tests which have been modified for purpose. The principle central to each recent HIV infection assay is that an optimal threshold and mean duration of recency are defined and used to determine whether or not an individual is likely to have been recently infected. These thresholds are determined by the individual OD, the quantified result of the anti-HIV immune response, as measured by the RITA assay, and the corresponding time since seroconversion for each person. Among a population of individuals with a known date of seroconversion, the OD and corresponding date of sample for each person is examined to establish the optimal threshold which will best define a recent infection. Through defining this threshold the mean time from seroconversion to when individuals are estimated to reach this threshold is established to determine the MDRI.

3.2.1 p24 antigen

The p24 antigen is one of the first serological markers present after initial exposure to HIV, and its evolution may be defined by three stages [114, 115]. Following initial exposure, a person will be negative for both HIV antibodies and p24 antigen (stage 1), then negative for HIV antibodies and positive for p24 antigen (stage 2) and, finally, test indeterminate for HIV antibodies and positive for p24 antigen (stage 3). After these three stages, the person may or may not be positive for p24 antigen but will test positive for HIV antibodies. Persons identified in either the second or third stage are classified as recently infected. Brookmeyer *et al* estimated the duration of stages 2 and 3 by examining serology from 15 patients who were positive for p24 antigen and negative/indeterminate for HIV antibodies and approximated stage 2 to last for 3.5 days and stage 3 for 19.0 days [115]. The main limitation of this method is the window period for detection which due to the short time period, requires the sample size for the study to be large and if possible from a population with high HIV incidence, ensuring a sufficient number of persons who have not yet seroconverted but have detectable p24 antigen are available.

3.2.2 HIV-RNA

Like the p24 antigen, the presence of HIV-RNA in the absence of HIV antibodies can be used to identify a recent HIV infection. Although, HIV-RNA is present throughout a person's infection, for an estimated 28 days after initial infection HIV-RNA is present in the absence of any immune response to the virus [116, 190]. Again like the p24 antigen, a large sample size is needed for estimating incidence due to the short window period when RNA is present in the absence of antibodies.

3.2.3 Sensitive/Less sensitive EIA or 'detuned'

The sensitive/less sensitive enzyme immunoassay (LS-EIA) relies on the ability to identify the rising concentrations of HIV antibodies using two versions of the Abbott 3A11 assay [117]. Through increased serum dilution (1:20,000) and shortened incubation times, the Abbott 3A11 assay was modified to be less sensitive to detecting lower concentrations of HIV antibodies. Persons who are reactive to the Abbott 3A11 and non-reactive to the less sensitive Abbott assay are identified as a recent infection. The OD or antibody concentration, for each sample is standardised using formula 3.1.

$$\frac{\text{(Sample OD - Negative control OD)}}{\text{Positive control OD}} \tag{3.1}$$

Both plasma and serum samples from 104 seroconverters, were collected from 3 sources and used to determine the duration of recency, with further samples collected from 268 persons infected for 2.5 years without AIDS and 158 with AIDS to determine how long the test remained reactive. The distribution of time between the last non-reactive results to the first reactive result for individuals were calculated using a mathematical model with varying cut-offs. The cut-off value found to be most reliable in estimating the time between tests was 0.75 with a duration of recency of 129 days (95% CI 109-149 days) (figure 3.1). Using this cut-off 0.4% of persons with a longstanding infection without AIDS and 2.4% of those with AIDS were misclassified as being recent.

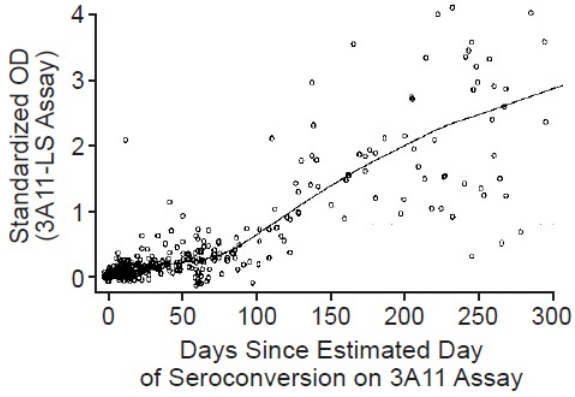


Figure 3.1: Distribution of seroconverters by days since seroconversion and optical density.

Source: Janssen *et al JAMA* 1998 [117]

Following the limitation of the Abbott 3A11-LS Enzyme Immunoassay (EIA) requiring specialized equipment and an FDA injunction in January 2000, a 2nd generation LS-EIA was developed, specifically the Vironostika-LS EIA [382]. Initial correlation measures were tested using the Abbott 3A11-LS EIA protocol, reporting a high degree of correlation between the Vironostika-LS EIA and 3A11-LS EIA, identifying recent from long standing specimens [382]. Further studies also found a high degree of correlation with >95% of specimens giving concordant results [282, 383], and in the UK, although a good correlation between the two assays was reported, the Vironostika-LS assay classified a higher number of specimens as recent [384]. More recently, a less-sensitive version of the diagnostic assay Vitros Anti-HIV 1+2 has been developed [385].

3.2.4 IgG BED CEIA

The BED-CEIA assay [118] measures the level of IgG antibodies in the person's serum, allowing the proportion of the total IgG which is HIV-specific to be calculated. As HIV-specific IgG is estimated to increase for two years post seroconversion, persons with a low proportion of HIV-specific IgG are likely to be recently infected. Unlike the LS-EIA, the BED-CEIA assay has the ability to detect HIV specific antibodies from a wider selection of subtypes, as it uses a branch peptide containing subtype B, E and D . The OD, the proportion of HIV-specific IgG, is calculated using formula 3.2.

$$\frac{\text{Mean Specimen OD}}{\text{Mean Calibrator OD}} \tag{3.2}$$

To define the optimal threshold and duration of recency for the BED assay, more than 600 longitudinal specimens with a known seroconversion date were tested, the distribution can be seen in figure 3.2. Sensitivity and specificity values were calculated for different assay and recency duration thresholds, increasing the assay threshold in 0.1 increments between 0.5 and 1.5 and the duration of recency in increments of 10 days between 120 and 220 days. With 81.7% sensitivity, and 89.1% specificity, the optimal thresholds identified were an OD of 1.0 within 160 days [118, 386]. Alternatively, the duration of recency was estimated as 158 days (95% CI: 151-163) using a linear mixed effect model.

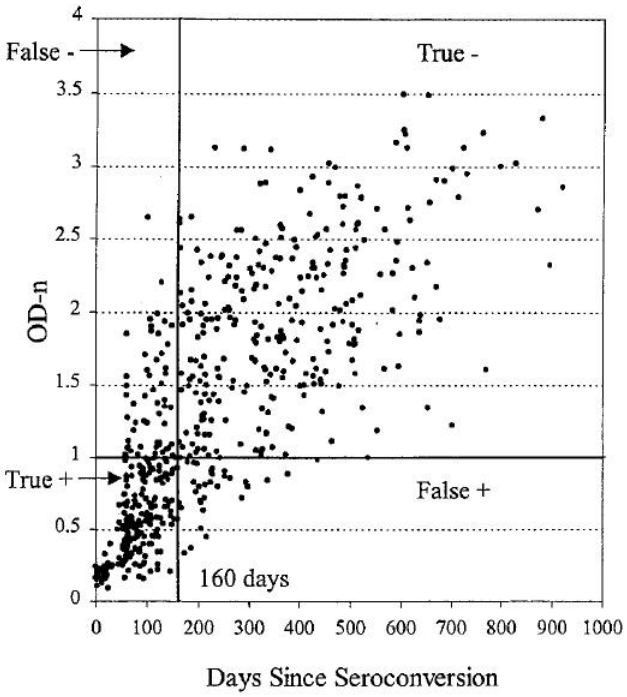


Figure 3.2: Distribution of specimens with known seroconversion, with suggested optimal thresholds.

Source: Parekh *et al AIDS Research and Human retroviruses* 2002 [118].

3.2.5 Avidity Index

The Avidity Index (AI), the strength of the bond between the viral protein (antigen) and the HIV-specific antibody, can be used for determining incident infections [119, 387, 388], a method already in use for the management of rubella and cytomegalovirus infection during pregnancy [Eggers 2000, Jenum 1997, Matter 1997]. Like the antibody response, the AI increases during the first year of infection, before stabilising at a value of 1, with antibodies having a low avidity for the antigen within the first six months of infection. The calculation for a person's AI is determined by formula 3.3.

$$\frac{\text{Sample cut off ratio of the guanidine aliquot}}{\text{(Sample cut off ratio of the phosphate - buffered saline aliquot)}} \tag{3.3}$$

The optimal threshold for distinguishing a recent infection was determined by using serum samples taken from 47 persons with a known date of seroconversion. Using the distribution of AI by time from seroconversion for these individuals (figure 3.3) a mean and median AI was identified to be lower than 0.8 for those diagnosed within 6 months from seroconversion. The diagnostic accuracy of the AI was investigated using ROC curves with a 79% sensitivity and 92% specificity for those with an AI <0.8 [119].

These findings were confirmed using a panel of samples for 1,075 days follow up, with an AI cut off of ≤ 0.75 equating to 125 days (85 to 164) and an AI cut off of ≤ 0.80 equating to 142 days (107 to 183) [387].

Since the initial application of the AI using the AxSYM HIV 1/2gO assay to differentiate recent from longstanding infections [119] there have been numerous applications, the two-well avidity index EIA (AI-EIA), single well limiting antigen avidity EIA (LAg-Avidity EIA) [389, 390], an avidity modified version of the Vitros Anti-HIV 1+2 assay [385], a modified version of the Bio-Rad Genetic Systems TM 1/2 plus O [391] and a bead-based multiplex assay using multiple avidity measurements from multiple HIV antigens [392].

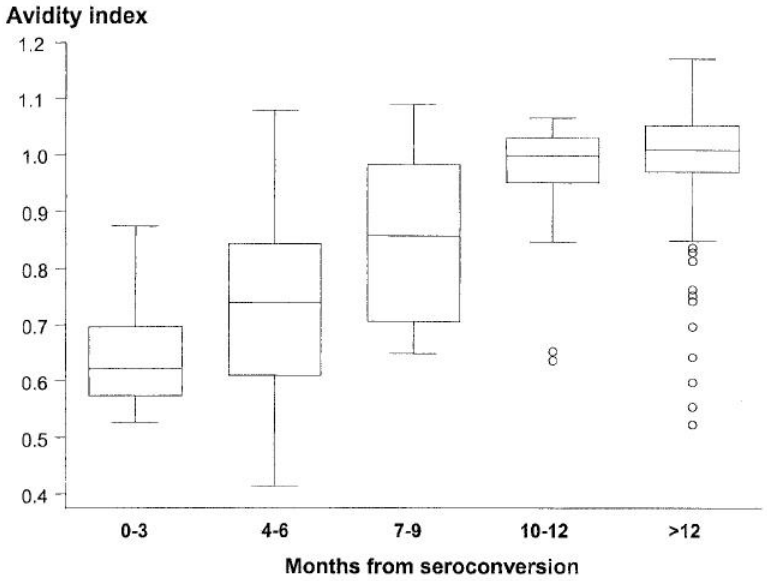


Figure 3.3: The distribution of AI by months from seroconversion for specimens with a known date of seroconversion.

Source: Suligoi *et al Journal of Clinical Microbiology* 2002 [119].

3.2.6 IDE-V3 EIA

The IDE-V3 EIA is a simple approach using an indirect enzyme-linked immunosorbent assay for identifying recent infections [120]. This assay uses the antibody concentration without the need of a commercially available assay; a limitation found with the avidity, detuned and BED assays. Four HIV antigens were identified based on particular features, a. whether the antigen was recognised in all patients, b. the antigen's clade relationship, and c. the ability to obtain and detect the antigen after seroconversion [120]. The four antigens chosen were the immunodominant eptitope (IDE) of gp41, V3 peptides, Integrase (IN) p32 and p24. Using serum for persons with known dates of infection, the antibody concentration was recorded for each of the four antigens, and used to determine whether there was a distinction between persons infected in the last 6 months (<180 days) and long standing infections (>180 days).

A definite distinction was identified in the median ratio for antibodies found in patients with a recent infection compared with established infections using the antigens IDE and V3, with a 20 fold difference for each [120]. The distinction between samples using IN and p24 were much lower (10 fold) but with considerable overlap between the two groups to allow meaningful separation between them (figure 3.4). Samples were classified into recent and long-standing infections using the probability of being classified as long-standing based on the combination of antigens. These probabilities were estimated using logistic regression with a probability of ≤ 0.5 being classified as recent and >0.5 being classified as long-standing [120].

All possible combinations of the different antigens were tested to determine the best model using ROC curves. The IDE alone or in combination with V3 was found to best differentiate between recent and long-standing infections. The sensitivity and specificity using the IDE-V3 combination was 88.3% and 100% respectively [120].

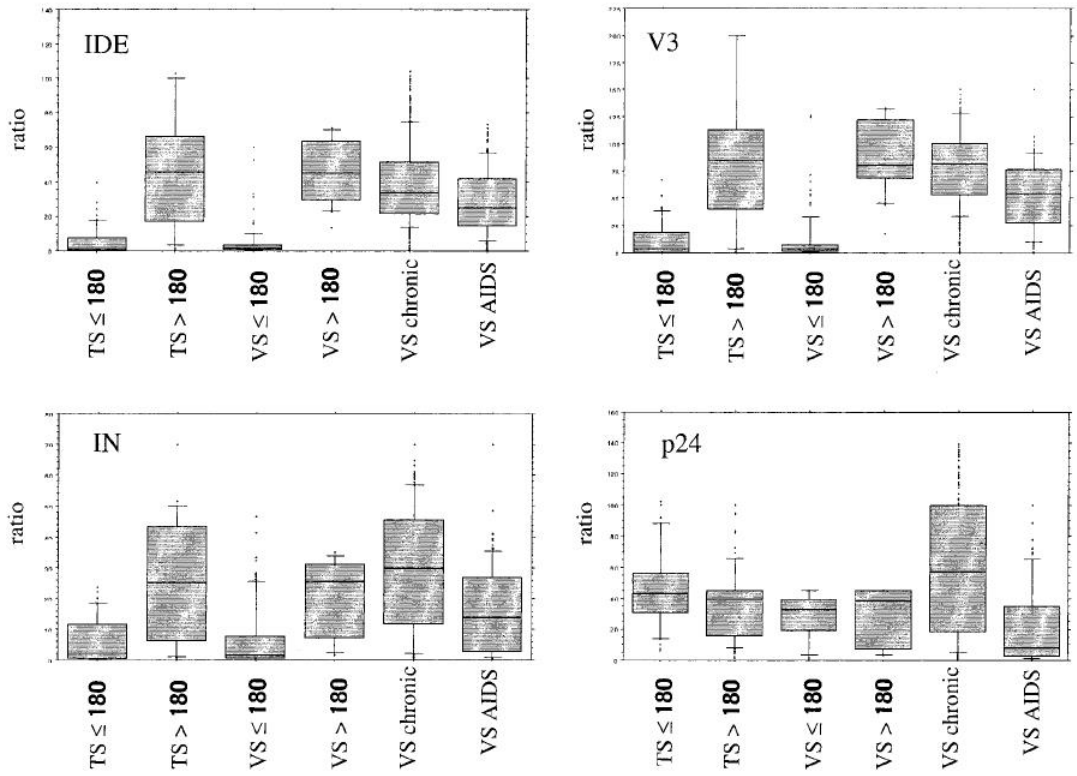


Figure 3.4: Probability distribution of being classified as recently infected (≤ 180 days) by antigen, among persons with a known date of seroconversion.

TS: Training Sample; VS: Validation sample; (VS used as an external validation of the parameters estimated in the TS). Comparison was made for less than or equal to 180 days vs. greater than 180 days and longstanding infections with (AIDS) vs. without (chronic) symptoms.

Source: Barin *et al Journal of Clinical Microbiology* 2005 [120].

3.2.7 IgG3 Isotype

Another simple approach is examining whether antibodies generated in response to specific antigens are indicative of a recent infection. This was achieved using an antibody isotype-specific HIV-1 western blot which was further quantified using an antibody isotype-specific enzyme-linked immunoabsorbant assay [121]. The response of three different biotinylated secondary isotyping antibodies (IgG, IgG1 and IgG3) to HIV-1 specific antigens among persons testing for HIV during and following seroconversion were examined. IgG and IgG1 resulted in a wide range of HIV-1 specific antigens being detected, whereas IgG3 identified responses to p17 and p24 with transient responses to p66 and p32 (figure 3.5). When the anti-p24 response was measured by densitometry it was found to be highest during 58 and 86 days after infection.

To quantify these results an IgG3-specific anti-p24 enzyme-linked immunoabsorbant assay was applied to 17 seroconversion panels. In agreement with the western blot results each of the 17 panels recorded a positive IgG3 reaction towards p24. Plotting absorbance units by time since seroconversion indicated that the optimal absorbance cut off of 0.5 would differentiate recent from long-standing infections within a recency period of 34 to 120 days [121].

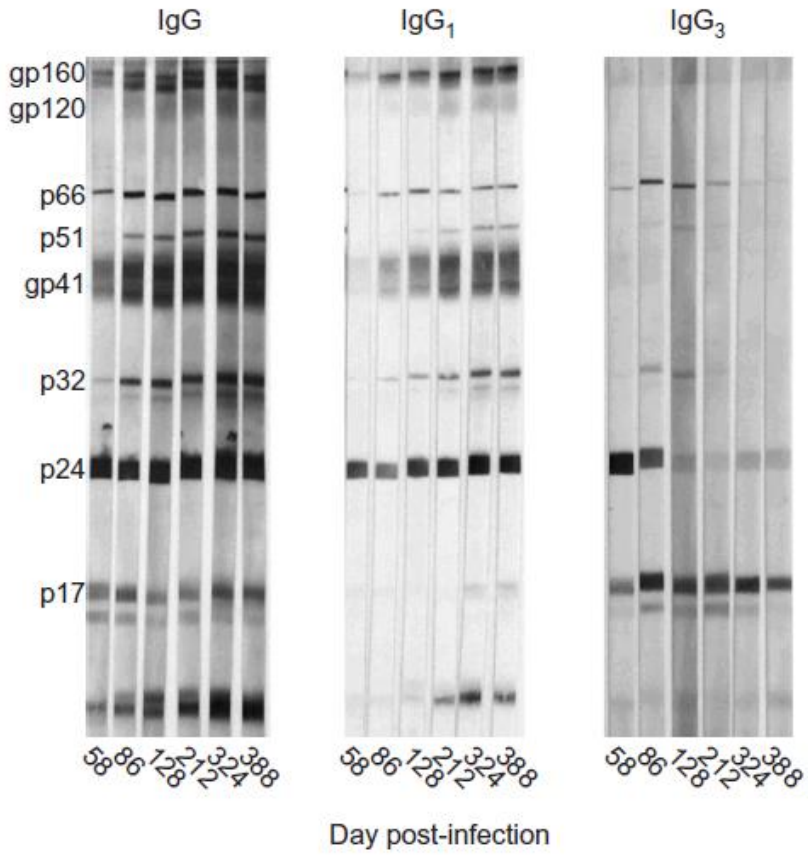


Figure 3.5: Distribution of the antibody isotype-specific response to HIV-1 antigens by time from infection among persons with a known date of seroconversion.

Source: Wilson *et al AIDS* 2004 [121].

3.2.8 INNO-LIA HIV I/II score

Another diagnostic tool modified to detect recent infections is the INNO-LIA HIV I/II score assay [122]. Like the IgG3 isotype, the INNO-LIA uses antibody reactions to seven HIV antigens, sgp120, gp41, p31, p24 and p17 for HIV-1, and sgp105 and gp36 for HIV-2, to determine a recent infection. A modified scoring system of the antibody reactions classified the results into one of six possible intensity scores (0, 0.5, 1, 2, 3 or 4). Using new diagnoses already classified as recent or long standing, through symptoms of primary infection, testing history or laboratory evidence of a seroconversion, different algorithms of INNO-LIA antibody patterns were investigated to determine which best identified a recent infection (figure 3.6).

For each of the five HIV-1 antigens a low antibody score was found to be associated with a recent infection. However, individually the antigens identified only a low proportion of recent infections correctly with low sensitivity scores. When using the antigen bands in combination, the proportion of recent infections correctly identified increased. The most successful algorithms were identified as 11-13, with a sensitivity score of between 40-50% and specificity score between 95-99%, using these algorithms 70% of recent infections were identified correctly which was comparable to the 72% identified when using the BED-CEIA [122].

Alg #	Algorithm Criteria
1	BED-EIA
2	sgp120 $\leq$ 1
3	gp41 $\leq$ 0.5
4	p31 = 0
5	p24 = 0
6	p17 = 0
7	sgp120+gp41+p31 $\leq$ 4
8	gp41 $\leq$ 0.5; *or* sgp120+gp41+p31 $\leq$ 4; *or* sgp120+gp41+p31+p24+p17 $\leq$ 6.5
9	sgp120+gp41 $\leq$ 4 *and* p31 = 0
10	p31=0 *and* p24 $\geq$ 2
11	sgp120+gp41 $\leq$ 2.5; *or* sgp120+gp41+p31+p24+p17 $\leq$ 6.5; *or* p31 = 0 *and* p24 $\geq$ 2
12	gp41 $\leq$ 0.5; *or* sgp120+gp41+p31 $\leq$ 4; *or* sgp120+gp41+p31+p24+p17 $\leq$ 6.5; *or* p31 = 0 *and* p24 $\geq$ 2
13	sgp120+gp41 $\leq$ 4 *and* p31 = 0; *or* p31 = 0 *and* p24 $\geq$ 2

Figure 3.6: INNO-LIA algorithms investigated to determine the most appropriate model for detecting recent infections.

Source: Schupbach *et al PloS ONE* 2007 [122].

3.3 Mean Duration of Recency

The MDRI is the time from seroconversion until a person is no longer defined as recent and can be estimated using the following methodologies:

- Linear Mixed Model (LMM) which estimates the slope and intercept for each person. The duration of recency is estimated as the time interval between the slope intercepting the OD baseline and theOD cut-off for the assay [118, 320].
- Non-Linear Mixed Model (NLMM) which uses the changing OD recorded at each observation. Using iterations from the Markov Chain Monte Carlo method the MDRI is estimated [393].
- Survival Analysis: The upper and lower bounds of recency duration are estimated using intervals for seroconversion and the OD cutoff [393].
- Graphical: A graphical representation of OD by time since seroconversion allows the sensitivity and specificity to be established by increasing the OD cutoff by time. This method is previously shown in figure 3.2 [118].
- Proportion of recent infections among seroconverters: Among a population initially negative at time 0 and then tested again at time T, the probability that an individual is recent equals MDRI/T, with the MDRI estimated as recent/positive at time T [394].

A comparison of the above methods indicated that theNLMM had the lowest coefficient of variation and the greatest variation was in the graphical method [395].

3.4 Additional Factors impacting serological tests.

There are a number of limitations to be considered when using any of the RITA assays which may be specific to one assay or applicable to all, as they may affect the accuracy of the incidence estimates.

3.4.1 Antibody Response

Using a RITA assay on a specimen from an individual with established infection such as an AIDS defining illness, or low CD4 cell count could result in them being misclassified as recent. These misclassifications are the result of decreasing antibodies due to a weakened immune system. Apart from the Avidity assay, all of the RITA assays differentiate recent from long-standing infections using the antibody response, with a low antibody response indicating a recent infection. However, low antibody responses are also made by individuals diagnosed with AIDS and low CD4 cell counts with rates of misclassification varying between assays. Studies where individuals with a current AIDS diagnosis were tested with a RITA assay reported the following rates of misclassification; for the Sensitive/Less sensitive assay, 2.9% of long-standing infections were identified as recent using the 3A11 Abbott [117] and 2.4% using the Vironostika-LS [383], for the BED-CEIA the rate was 2-3% [396] and for the IDE-V3 9% [120].

In addition, persons taking ART, and therefore with low/undetectable viral load, are likely to be misclassified as recent [397]. This is due to the suppression of the viral replication resulting in a low antibody response, much like persons with an established infection. When using the IDE-V3 on samples from individuals treated with ART, the majority of samples which were known to be from established infections were classified as recent [120]. Individuals on treatment for two years or more were more likely to be misclassified as recent [397–399]. In two studies, the proportion misclassified increased from 12.4% among treatment naive patients to 23.8% among those on treatment for two years [397], and 11.2% to 56% respectively [399]. Further work suggests that ART impacts the evolution of the AI in persons immediately taking up treatment by minimising viral replication. In a small study of 13 persons with primary HIV infection, the AI in those untreated increased whereas for those on treatment the AI was stable [400].

3.4.2 HIV Subtype

Although the subtype an individual is infected with does not directly affect the RITA assay, it is known to influence the recency period (the period which an individual would be classified as recent). Two studies comparing Subtype B with E, found in both cases that subtype B had a shorter recency period than E, 155 days (125-189) vs. 270 days (187-349) [128] and 239 days (208-287) vs. 356 days (318 vs. 402) [130]. The BED-CEIA attempts to minimise the impact of different subtypes by using a branched peptide containing subtype B, E and D [118]. A further study investigating seroconverter panels for subtypes A/D, B, C and E identifed that although subtype B and E had shorter recency periods the 95% confidence intervals for all the subtypes investigated overlapped [128, 401, 402] (Figure 3.7).

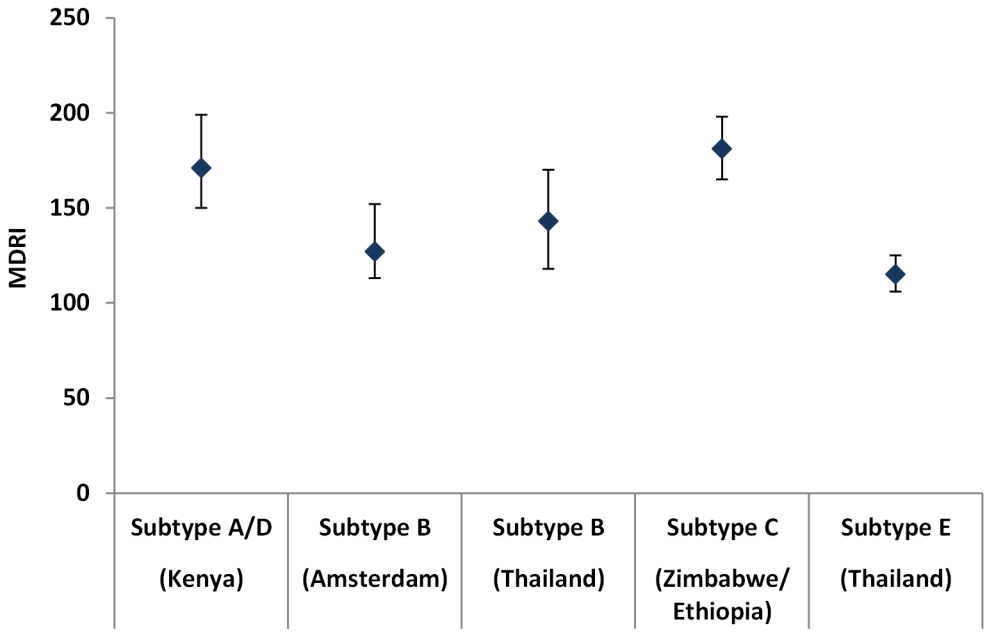


Figure 3.7: Window Period for the BED-CEIA (0.8 cutoff) by HIV subtype.

Source: Parekh *et al* HIV Diagnostics: New Developments and challenges 2005 [402].

3.4.3 False Recency Rate

Aside from AIDS, low CD4 count and treatment, a number of individuals maintain an optical density below the assays threshold for long periods following seroconversion, and some may never rise above it. This fraction of treatment-naive persons with long-standing HIV infection but whom the assay identifies as being recently infected is known as the False Recent Rate (FRR). This fraction is obtained from panels of well characterised specimens from individuals with long-standing infection from the population for which incidence is to be estimated, and used to correct for misclassification (discussed in chapter 4). Where additional clinical information on AIDS, CD4 or treatment is not available for reclassifying recent infections as long-standing, the FRR can be estimated for all individuals. In settings where it is not feasible to calculate the FRR, samples from similar populations can be used, however as the FRR can differ significantly across settings resulting in very different incidence estimates, careful consideration must be given when identifying an appropriate FRR [125, 403].

3.5 Sampling Strategy

There are 3 main frameworks used for estimating incidence: a cross-sectional sample, sentinel surveillance and case-reporting through routine testing sites.

Cross-sectional studies collect data on a study population, representative of the whole population at a given point in time. Persons are included using a sampling strategy or through convenience sampling based on accessibility using sexual health clinics, bars or community groups. Cross-sectional studies are a quick and easy method of collecting specimens with limited follow-up time needed. However, using convenience sampling may not necessarily be representative of the whole population, makes it difficult to investigate incidence over time, and difficulties arise in capturing hard to reach populations such as CSWs or PWID.

Sentinel surveillance allows detailed information to be collected from a limited number of sites, which could be a population of particular interest or a representative sample of the overall population. This methodology is less expensive with high quality data due to the focused design. However, like using convenience sampling for cross-sectional studies, sentinel surveillance may not be representative of the overall population. Methods for recruiting individuals may also

influence incidence estimates with persons testing due to high risk behaviour or symptoms making it difficult for results to be generalisable beyond the survey population, and leading to an overestimate of incidence.

Both cross-sectional studies and sentinel surveillance methodologies collect information on both the sero-negative and positive population allowing HIV incidence to be estimated using the approach described in section 4.2.

RITA testing can also be introduced into already established surveillance systems, developing a better understanding of the current epidemic, allowing for temporal trends. However, changes in testing behaviour through campaigns to promote testing will introduce bias in reporting of HIV diagnoses, alongside persons seeking a test due to high-risk behaviour or symptoms of primary infection inflating incidence estimates.

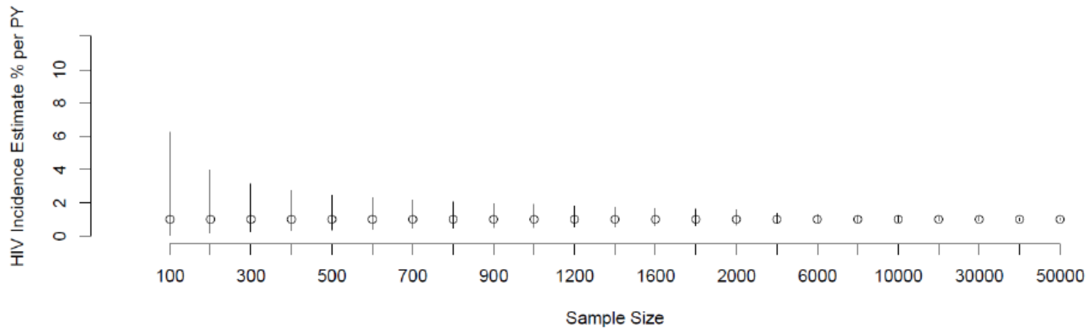
For the majority of studies using surveillance systems to identify recent infections, information on only persons testing positive will be available. As the sero-negative population is not available, an alternative method for estimating HIV incidence is described in section 4.3.

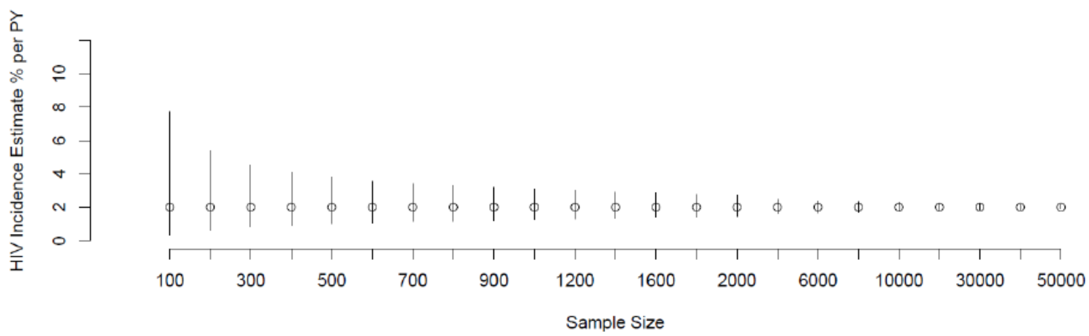
3.6 Sample Size

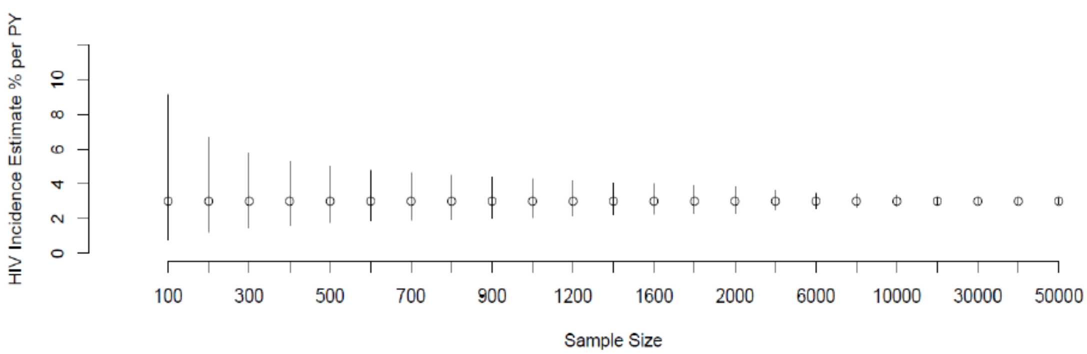
Incidence estimates are dependent on the number of persons included within the study, where the sample size is generally larger than would be needed to estimate prevalence [404]. Sample sizes are based on the anticipated incidence and estimated prevalence for the population of interest, the duration of recency for the assay used and the expected proportion of false positives among those with a long-standing infection, also known as the FRR [405]. This method estimates the sample size required in a cross-sectional framework and includes data from both positive and negative individuals. When introducing RITA as part of existing surveillance schemes, in many cases the only information available will be on the HIV positive population. Centre for Disease Control (CDC) recommends 300 HIV positive cases within the selected period and at least 200 cases per stratum with 40 cases specimens tested and at least 10 recently infected [Daniela De Angelis, Personal Communication, August 2013]. Where cost limitations arise, consideration should be given to the sample size and sampling methodology.

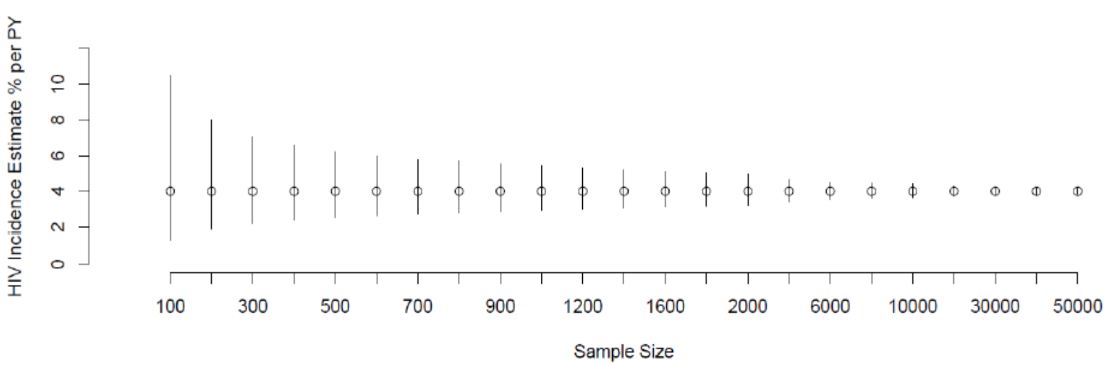
A key element of the incidence formula, using existing surveillance schemes, is estimating the probability of testing within a year of diagnosis. This is achieved using either a patients testing history where available or the testing frequencies of the population. These probabilities are likely to differ for subpopulations such as age, sex or by risk factors, resulting in incidence estimates being calculated for stratified populations. The sample size and sampling methodology is therefore equally important to be considered for these subpopulations.

In the series of graphs within figure 3.8 the effect sample size has on the confidence intervals for HIV incidence using the cross-sectional methodology is illustrated. The five graphs show the effect of between 1 and 5% HIV incidence on samples sizes ranging from 100 to 50,000 persons. As the sample size increases, so does the level of certainty.


HIV Incidence Estimate % per PY
0 2 4 6 8 10
100 300 500 700 900 1200 1600 2000 6000 10000 30000 50000
Sample Size


HIV Incidence Estimate % per PY
0 2 4 6 8 10
100 300 500 700 900 1200 1600 2000 6000 10000 30000 50000
Sample Size


HIV Incidence Estimate % per PY
0 2 4 6 8 10
100 300 500 700 900 1200 1600 2000 6000 10000 30000 50000
Sample Size


HIV Incidence Estimate % per PY
0 2 4 6 8 10
100 300 500 700 900 1200 1600 2000 6000 10000 30000 50000
Sample Size

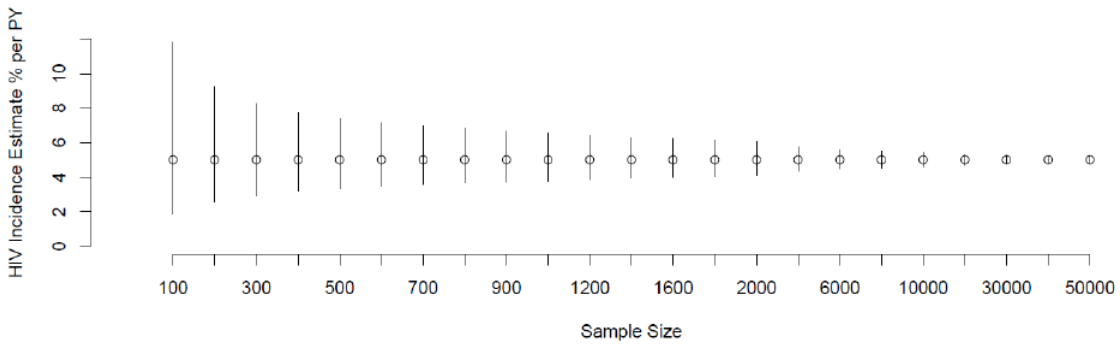


Figure 3.8: Effect of sample size on confidence intervals for HIV incidence estimates of between 1 and 5%.

Source: European Centre for Disease Prevention and Control/WHO Regional Office for Europe 2013 [406].

3.7 Summary

The distinct maturing anti-body response to HIV has enabled laboratory methods to be developed to differentiate a recent from longstanding infection, requiring only a single diagnostic sample taken at a single point in time. Currently, there are eight assays developed and, although these methodologies use different biological markers, they all require a person to be tested within a specified time after infection which is specific to the assay being used.

Initial methodology introduced used the presence of p24 antigen or HIV RNA in the absence of HIV antibodies among individuals who had not yet seroconverted. The major limitation to this was the short duration of recency, which required a large sample size to calculate incidence estimates. Alternatively, assays for post seroconversion were introduced using titre, antibody or avidity cut-offs or reactions between specified antigens and antibodies. Each of these biological markers has been developed with the aim of differentiating recent from long-standing infections. However, there are distinct advantages and disadvantages which need to be considered when designing an incidence study. The detuned assay has limited use beyond subtype B populations, the INNO-LIA is expensive if not routinely used, whereas the IDE-V3 aand IgG3 are both inhouse methodologies but are not yet commercially available. In addition, the IDE-V3 has a much lower sensitivity than other assays and the IgG3 is still to be validated for use with different subtypes. Finally, there is a limitation to their use because of misclassification of long-standing infection as recent. This is particularly high for the BED assay. Although the avidity assay is also prone to misclassification, it is much lower than has been found for the BED assay, is also commercially available, and requires no additional laboratory equipment.

Addressing these limitations, particularly the misclassification of long-standing results as recent, is imperative to ensure appropriate incidence estimates are made. The misclassification of results is applicable to all assays, although with differing intensities. Using the assay in conjunction with clinical information ensures individuals who have low antibody responses due to AIDS or use of antiretrovirals are correctly classified as long-standing. Additionally, the FRR, the fraction of treatment-naive individuals known to have a long-standing infection identified as recent, is used to correct for misclassified results as part of the formula used to identify the true population recently infected (see section 4.4.2) . This fraction can be used in addition to clinical information for persons who will maintain an OD below the threshold for long periods

of time with no evidence of treatment or AIDS or for all persons where clinical information is not available. Importantly, where possible the FRR should be made among the population of interest or a similar population.

Finally, the correct sampling framework is also essential, ensuring that samples tested are representative of the overall populations from which they are drawn. Appropriate sample sizes should be calculated before initiating testing and consideration should be made in regards to the population being sampled so the most appropriate assay is chosen.

Chapter 4

Statistical Methods for Estimating HIV Incidence using Serological Tests

Within the following chapter, I present the different statistical methods used to estimate incidence when using a serological test for recent infection, with both direct and indirect methodologies to minimise the chance of misclassified results.

These methods include using a cross-sectional framework, focusing on a purposely recruited population, or on data collected through national surveillance among a population choosing or referred for testing.

4.1 Introduction

The statistical methodology used to estimate incidence is dependent on the sampling framework used. However, the two key elements used in each methodology are:

- the number (or proportion) of sero-positives which are recent, and
- the duration of recency of the assay being used.

There are two fundamentally different designs. The first design, a cross-sectional framework, relies on data from a purposely-recruited probability sample of the population of interest. These data include both HIV positive and negative individuals and may be directly generalisable to the overall population. By contrast, the second design, introducing a test for recent infection to HIV diagnoses only, relies on data from a population choosing to test, or being targeted for testing, where only information on the sero-positive population is available. Incidence estimates for a population undergoing HIV testing will be impacted by testing frequencies and testing motivation. Symptoms of primary infection, high risk behaviours and concurrent Sexually Transmitted Infection (STI) symptoms could lead to increases in testing rates and at earlier time points [407, 408].

Alongside choosing the appropriate methodology for the sampling framework, additional considerations must be made to minimise the impact of misclassified results. Within this chapter, through literature identified in the methods section of chapter 2, I will describe the different methods for estimating incidence and discuss modifications to minimise the effects of specific limitations.

4.2 Estimating incidence using a cross-sectional framework

The cross-sectional framework requires population-based data with information from both the sero-negative and positive population and is directly generalisable to the population sampled. Populations where such information is regularly collated are antenatal screening and blood donors.

The following terms/notations will be used within section 4.2.

N	Total Population
I	HIV Incidence rate
D	Duration of disease
N_r	Number classified as recent
N_n	Number HIV negative
P	HIV Prevalence
μ	Assay recency period (days)

We start with the relationship between incidence and prevalence, where prevalence can be determined by the incidence rate and the duration of the disease, as seen in formula 4.1. This interrelationship assumes that incidence is constant as is the duration of the disease. Any one of these three measures can be derived when the other two measures are known.

$$P \approx I \times D \tag{4.1}$$

Using this interrelationship with the assumption that the rate infected during the recency period is constant, Brookmeyer and Quinn used formula 4.2 to estimate incidence [114]. To determine incidence per year, the proportion of persons classified as recent within the recency period is multiplied by a correction factor (365/recency period). Brookmeyer and Quinn used the presence of p24 antigen in the absence of antibodies, which occurs on average 22.5 days before seroconversion [114]. Therefore, the estimated incidence per-cent per year is equal to the prevalence of recent infections (occurring 22.5 days before seroconversion) multiplied by the correction factor 16.5 (365/22.5).

$$(\frac{N_{\mathrm{r}}}{N_{\mathrm{r}}+N_{\mathrm{n}}})\times(\frac{365}{\mu})\times 100 \tag{4.2}$$

Brookmeyer and Quinn [114], identified 15 persons of the 1241 seronegative who attended an STI clinic in Pune, India between May 1993 and January 1994 and were p24 antigen positive, resulting in an estimate of 19.6 percent per year.

$$(\frac{15}{1241})\times(\frac{365}{22.5})\times 100$$
$$=19.6 percent per year$$

Formula 4.2 was also used by Janssen *et al* [117] for the sensitive/less sensitive assay where the duration of recency was 129 days resulting in a correction factor of 2.83. Among persons donating blood for the first time between 1993 and 1996 in 32 American Red Cross collection regions, 69 of the 2 717 910 tested were identified as recent (478 were HIV positive but not recent), resulting in an incidence estimate of 7.18 per 100 000 per year.

$$(\frac{69}{2717432})\times(\frac{365}{129})\times 100$$
$$=0.00718 percent per year$$

A further variation of formula 4.2 was applied by Parekh *et al* [118] when using the BED-CEIA assay (section 3.2.4). Within formula 4.3 the correction factor for determining the number of recent infections classified within a year is applied to the number classified as recent directly. The recency period for the BED-CEIA assay is 160 days, with a correction factor of 2.28 (365/160). The number of recent infections within a year is then divided by those at risk of HIV (including both negative and recently infected persons) resulting in the incidence per-cent per year.

$$\frac{[(\frac{365}{\mu})\times N_{\mathrm{r}}]}{[N_{\mathrm{n}}+((\frac{365}{\mu})\times N_{\mathrm{r}})]}\times 100 \tag{4.3}$$

Using data from Janssen *et al* [117] the following equation results in an incidence estimate of 5.8 per 100 000 per year.

$$\frac{[(\frac{365}{160}) \times 69]}{[2717432 + ((\frac{365}{160}) \times 69)]} \times 100$$

$$= 0.0058 percent per year.$$

4.3 Estimating incidence using sero-positives

Using data from sero-positives to estimate incidence differs from the cross-sectional framework in two major areas. Whereas, for the cross-sectional method, data are available for both the infected and uninfected populations, only data for those infected are available. Consequently, the number of persons at risk which is used as the denominator within the cross-sectional formula is not available. Furthermore, diagnostic reporting represents a population selected for testing or choosing to test compared with a sample from a population recruited into a study resulting in different testing frequencies among subpopulations. As a result, an alternative method was introduced by Karon *et al* [409].

4.3.1 Inital approach to estimating incidence using sero-positives

The following terms/notations will be used within section 4.3.

N	Total Population
I	Incidence rate
t	Reference Period (Duration of interest)
N_r	Number classified as recent
T_r	True number recent
μ	Assay recency period (days)
α_A	Shape parameter of the incubation period from infection to an AIDS diagnosis
β_A	Scale parameter of the incubation period from infection to an AIDS diagnosis
q	proportion of persons diagnosed with HIV and AIDS simultaneously
P	Probability of testing and being classified as recent
P_1	probability of having a HIV test within the reference period (t-year)
P_2	probability of a confirmed positive sample having a RITA result
P_w	probability of being classified as recent (within μ)

There are multiple variations to consider, the reference period throughout this chapter will refer to 365 days and is assumed to be at least as long as the recency period. P_w varies depending on the assay being used and P_1 will differ from person to person. As each person has a unique P_1, P can be calculated separately for each person or as an average. There are also numerous

assumptions to consider.

- Date of HIV infection and date of HIV test are independent.
- The test for recent infection is conducted on the sample obtained at the time of new diagnosis.
- For persons with a previous negative date the infection date has a uniform distribution between last negative and first positive test.
- Persons testing for the first time have a constant testing hazard after infection, and the recency period and time from infection until AIDS diagnosis are independent.
- For repeat testers the testing behaviour has not changed during recent years.

The true number of recent infections within the population can be estimated by a function of the probability that an individual is tested and classified as recent (P). This method for estimating incidence has been adopted by both the United States [345, 346, 409, 410], France [349] and Italy [[348] using the underlying formula 4.4.

$$T_{\mathrm{r}} = N_{\mathrm{r}} / P \tag{4.4}$$

In the formula the number of detected recent infections (N_r) equates to the number of HIV positive individuals who have come forward for a diagnostic test and have been tested with a test for recent infection assay and for whom the test indicated a recent infection. To establish the true number of recent infections within the population (T_r), the number of diagnostic tests identified as recently infected is divided by the probability of being tested and classified as recent (P), which is assumed constant across individuals or the average value is used.

For example, Karon *et al* [409] identified 908 recent infections and estimated P (the probability of testing and being classified as recent) as 0.105, resulting in the true number of recent infections estimated as 8648.

$$908/0.105$$

$$= 8648$$

The first method of estimating the probability of detection was calculated using three component probabilities [409].

- P_1 probability of having an HIV test within the reference period
- P_2 probability a confirmed positive sample has a RITA result
- P_w probability of being within the recency period at the time of the RITA test

$$P = P_1 \times P_2 \times P_w \tag{4.5}$$

Further methods estimated P using only P_1 and P_w assuming P_2 is equal to 1 as a result of complete reporting of BED results or through imputation of the data [345, 349].

$$P = P_1 \times P_w \tag{4.6}$$

The probability that an individual has a RITA result classifying them as a recent infection within a year of infection (P_w) is estimated using formula 4.7, with the reference period, in this case 365 days, over the recency period. This equation assumes that the rate of new infections has been steady over the reference period.

$$P_w = \mu/365 \tag{4.7}$$

The probability of a person being tested within the reference period (P_1) is estimated separately within three strata:

- persons with a known previous negative HIV test,
- persons with no testing history and newly diagnosed with symptoms (such as an AIDS defining condition), and

- persons with no testing history and newly diagnosed without symptoms.

Persons with a known previous negative HIV test

The probability of testing within the reference period (P_1) is estimated based on the time from the person's last negative test to their first positive HIV test (T^*) in years. The probability that an individual's first positive test is within the reference period is estimated for each individual as $\min(1,1/T^*)$. All individuals are included regardless of whether they present for an HIV test due to symptoms or not. Table 4.1, shows examples of P_1 for repeat testers.

Diagnosis Date	Date of last negative test	Difference	P_1
14/08/2013	15/07/2004	9.1	0.11
27/09/2013	01/12/2008	4.8	0.21
04/04/2013	15/03/2000	13.1	0.08
17/09/2013	01/02/2013	0.62	1
07/10/2013	01/09/2011	1.1	0.91

Table 4.1: Example of the estimated probabilities for repeat testers based on the time between the last negative and first positive test.

Persons with no testing history

The probability of testing within the reference period (P_1), for persons without testing history is much more problematic and is estimated assuming a constant rate of testing.

An individual's first test after seroconversion can arise in either of two ways, and we denote the time till the test by T_1.

- T_R the time from seroconversion to an individual coming forward at random for an HIV test
- T_A the time to testing due to an AIDS defining illness

T_1 is therefore equal to the minimum of these two times, min(T_A,T_R). For a person to be tested within the recency period T_1 must be less than the recency period.

If T_1= T_A, diagnosed with both HIV and AIDS simultaneously, then T_1 is the incubation period in years (from infection to AIDS), which is assumed to follow a distribution determined using Markov models, modelling the different stages or biological markers of a person's infection.

Alternatively, if T_1= T_R it is hypothesised that an individual's first test would have occurred during the incubation period prior to symptoms. To estimate the distribution of T_R we assume an individual tests for HIV randomly over this time period following an exponential distribution with the scale parameter defined as.

$$1/\beta \tag{4.8}$$

To determine β, the shape (α_A) and scale (β_A) parameters of the incubation period until an AIDS diagnosis, assuming a gamma distribution and the proportion of persons for whom T_1=T_A, are required, a shape parameter of 2 and a scale parameter of 4 was proposed by Karon *et al* [409],and specifically the scale parameter is taken to be:

$$\beta = \frac{[\beta_A]}{[q^{-1/\alpha_A} - 1]} \tag{4.9}$$

For example within a population where 20% of persons are newly diagnosed with a simultaneous HIV and AIDS diagnosis, β = 3.2, with the estimated mean time from infection to first test is therefore 3 years and the probability of testing within a year of infection is 31% (formula 4.10).

$$\beta = \frac{[4]}{[0.2^{-1/2} - 1]}$$

$$= 3.2 \tag{4.10}$$

$$1/\beta = 0.31$$

For a population with 5% newly diagnosed with a simultaneous HIV and AIDS diagnosis, the time from infection to first test is 1 year or less with a much higher probability of 87% (formula 4.11).

$$\beta = \frac{[4]}{[0.05^{-1/2} - 1]}$$

$$= 1.15 \tag{4.11}$$

$$1/\beta = 0.87$$

4.3.2 Modified approach to estimating incidence using sero-positives

The original calculation of the probability of testing and being classified as recent (P) was further modified by Prejean *et al* in 2011. These modified methods for estimating P, allow for frequent testers whose time between testing might be less than a year and considers that the duration of recency for an individual could be greater than 1 year. As these assumptions are not considered by the Karon methodology [409] the probability of testing within the reference period is bounded by 1. Table 4.2 shows the differences in the probability of testing within a year of infection by the two methodologies, where Prejean *et al* consider increasing time from negative test to positive test in months, Karon *et al* consider this time in years. By considering that an individual will test at yearly intervals a lower probability is estimated than if the time between tests is less than a year, which leads to an overestimation of the number of true recent infections.

Interval between negative (month or year) and positive test	**Probability of testing within a year of infection (where interval is months)**	**Probability of testing within a year of infection (where interval is years)**
1	0.996	1
2	0.984	0.50
3	0.937	0.33
4	0.869	0.25
5	0.794	0.20
6	0.726	0.167
7	0.662	0.143
8	0.603	0.125
9	0.550	0.110
10	0.502	0.100
11	0.462	0.091
12	0.428	0.083
13	0.398	0.077
14	0.372	0.071
15	0.349	0.067
16	0.329	0.063
17	0.310	0.059
18	0.294	0.056
19	0.279	0.053
20	0.265	0.050
21	0.253	0.048
22	0.242	0.045
23	0.231	0.043
24	0.444 X 12/(difference in months)	0.042

Source: Prejean *et al PLoS ONE* 2011 [346].

Table 4.2: Probabilities of testing within the reference period by HIV test interval (year or month) between last negative and first positive test, for repeat testers.

Furthermore, the overall probability (P) is bounded by the probability of being classified as recent (P_w) estimated by the recency period over the reference period (figure 4.1). Figure 4.1, shows this limitation by comparing the expected probability of testing and being classified as recent with increasing time since a person's last negative test using the Prejean method (blue line) with the calculated probability using P_w, Karon's method, where P is bounded by P_w estimated as $\mu/365$. As a result, P is calculated directly as follows, preventing an underestimation of P.

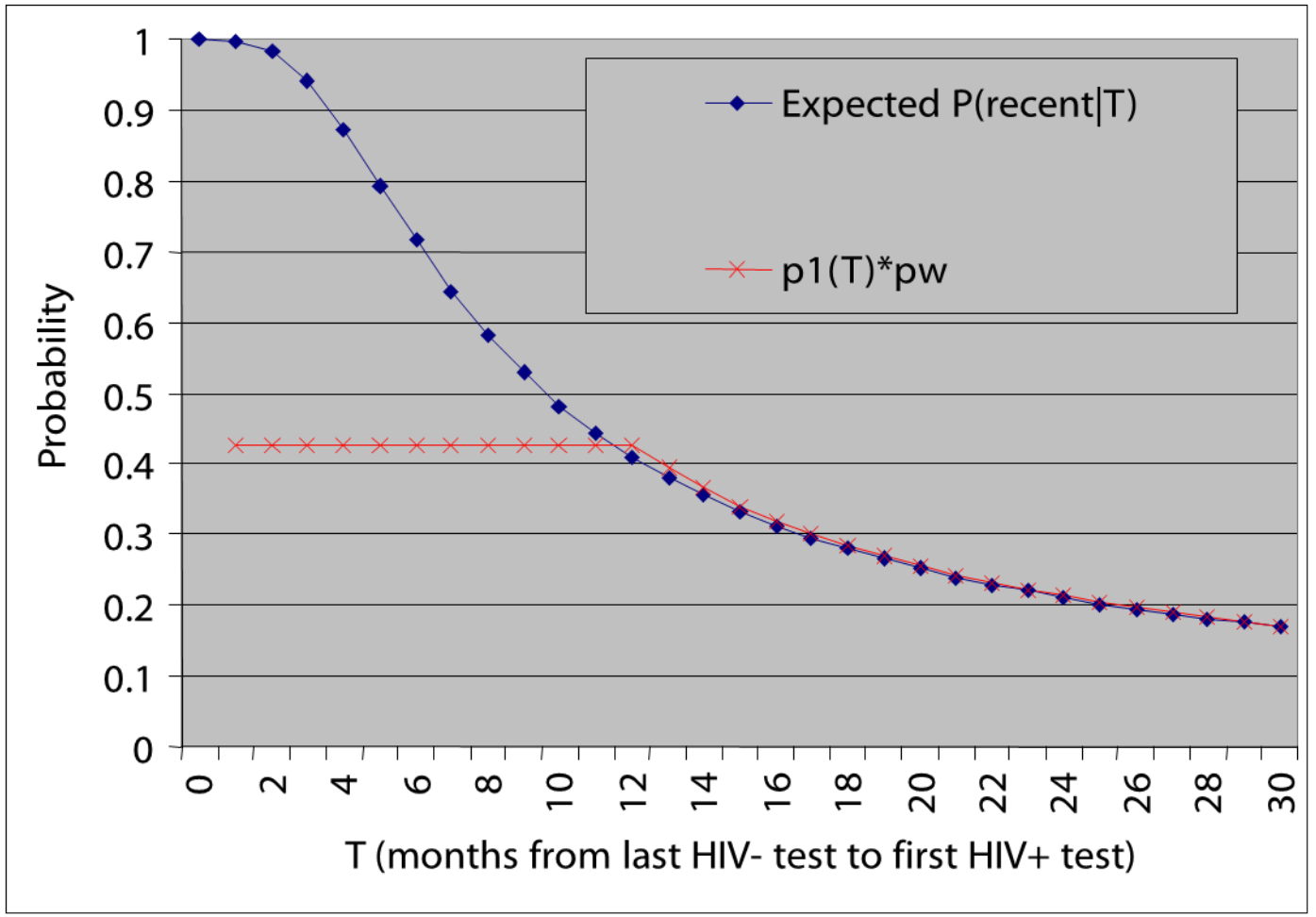


Figure 4.1: Potential bias using the simplified method for estimating the probability of testing and being classified as recent (P).
The red line represents the probability of testing within a year of infection with increasing time since the last HIV negative result as defined by Karon *et al* [409], whereas the blue line represents the probability of testing and being classified as recent using the modified method by Prejean *et al* [346]. Source: Rulguang Song, Personal communication, 23/08/2014.

The following terms/notations will be used within section 4.3.2.

P_i	Probability of testing and being classified as recent for person i
P	Average probability of testing and being classified as recent across all repeat testers
n	Number of repeat testers
T_i	number of months between last negative and first positive test for person i
Sw(t)	Probability of a person being classified as recent at time t after infection
$S_A(t)$	Survival function for the AIDS incubation period (as previously discussed)

Persons with a known previous negative HIV test (Direct Calculation)

For each person, P_i is estimated as the probability of a person being classified as recent (Sw(t)) at the time of their test since infection, over the distribution of possible infection time, which is assumed uniform between the last negative and first positive test. For each individual we consider the time in months between their last negative test and first positive test and assume that their date of seroconversion in uniformly distributed within this time frame. The probability of testing and being classified as recent for that individual (P_i) is the sum of each possible date of seroconversion within the time frame between the negative and positive date. The overall probability of testing and classified as recent (P) is then the average of each person's P_i. Therefore, if a person had a positive test 10 months after their last negative test, we can assume that the date of seroconversion occurred at some point during those 10 months, the probability of testing and being classified as recent is then estimated for each of the 10 months and the overall P_i the sum of these 10 probabilities.

$$P_i = \frac{1}{T_i} \int_0^{T_i} Sw(t)\,dt \tag{4.12}$$

4.3.3 Persons with no testing history (Direct Calculation)

The assumption for first-time testers is the same as previously with a constant testing rate until AIDS from infection, where the rate can be estimated based on the proportion of persons

diagnosed with a simultaneous HIV and AIDS diagnosis referred to as q.

$$\beta = \frac{[\beta_A]}{[q^{-1/\alpha_A} - 1]} \tag{4.13}$$

P is estimated by the weighted sum of the probability that a person tests within the recency period and the probability that the test occurs before AIDS.

$$P = \int_0^\infty Sw(t) S_{\mathrm{A}}(t) \frac{1}{\beta} e^{-\mathrm{t}/\beta} dt \tag{4.14}$$

Table 4.3 lists estimated probabilities by proportion testing late as published by Prejean *et al.*

Proportion of persons diagnosed simultaneously with HIV and AIDS	Probability of testing within a year of infection	Proportion of persons diagnosed simultaneously with HIV and AIDS	Probability of testing within a year of infection
0.01	0.575	0.26	0.099
0.02	0.452	0.27	0.095
0.03	0.383	0.28	0.092
0.04	0.336	0.29	0.089
0.05	0.301	0.30	0.086
0.06	0.274	0.31	0.083
0.07	0.252	0.32	0.080
0.08	0.234	0.33	0.077
0.09	0.218	0.34	0.075
0.10	0.204	0.35	0.072
0.11	0.192	0.36	0.070
0.12	0.182	0.37	0.068
0.13	0.172	0.38	0.066
0.14	0.163	0.39	0.063
0.15	0.156	0.40	0.061
0.16	0.148	0.41	0.059
0.17	0.142	0.42	0.058
0.18	0.136	0.43	0.056
0.19	0.130	0.44	0.054
0.20	0.125	0.45	0.052
0.21	0.120	0.46	0.051
0.22	0.115	0.47	0.049
0.23	0.111	0.48	0.047
0.24	0.106	0.49	0.046
0.25	0.103	0.50	0.044

Source: Prejean *et al PLoS ONE* 2011 [346].

Table 4.3: Probabilities of testing within the reference period by proportion of persons diagnosed simultaneously with HIV and AIDS.

4.3.4 Confidence Intervals

Two methods have been used to determine confidence intervals for incidence estimates using the sero-positive method, bootstrapping [348, 411] and the delta method [346, 349, 409]. The bootstrapping methodology resamples the original data to create a defined number of samples from which the outcome is estimated and the range of these estimates defined. For these data both the interval from the last negative and first positive test for repeat testers and the CD4 count at diagnosis for first-time testers are resampled 1000 times to produce 1000 probabilities required for each sample population. These 1000 probabilities give a range within which we can be 95% confident the true probability will lie. Whereas for the delta method the derivation of the incidence estimator variance, var(log(incidence)), is obtained and used to approximate the variance of a function, in this case 1/x, of one or more random variables in terms of their respective variances and covariance.

4.3.5 Testing Patterns

When using sero-positive data to estimate incidence, the main component is presenting for an HIV test. Therefore, differences in testing frequencies will result in different estimates of incidence with populations testing more frequently having a higher probability of testing within the reference period than those testing less frequency. As these probabilities inform the true number of recent infections within a population, an incorrect probability will result in over or under estimating incidence. To correct for heterogeneous testing patterns, when investigating subpopulations the methods described in section 4.3 should be applied to each sub-population of interest. Sub-populations to consider are age groups, sex and exposure category.

Previous literature indicated high rates of repeat testers among MSM and PWID [271, 412], persons participating in high risk behaviours [271, 407, 408, 412, 413] and older individuals [412, 414]. Four studies investigated testing among sexual health clinic attendees [414–416] and attendees of a same day HIV testing clinic [417] in the USA and the UK. Among attendees of a sexually transmitted disease clinic in the United States, a third of individuals had had a previous HIV negative test [414]. Among attendees of a same day HIV testing clinic in the UK, one in four

MSM and two in five heterosexuals were repeat testers [413]. Investigating MSM specifically, two studies identified that MSM had, on average, 1.6 tests per year, with 43% having more than 1 test within one year [416], and a third re-attend within a year for a HIV test, with 50% of re-attendees retesting within 12 months and 75% retesting within in 24 months [415]. Among PWID, data from the USA reported tha 49% had a previous negative test [417]. In Canada 56% had an HIV test within the past 12 months [418].

4.4 Minimising False Recent Infections

In section 3.4, I discuss the limitations to using RITA to estimate incidence with the possibility of long standing infections being classified as recent. These misclassified results occur either due to factors impacting the immune response (AIDS, low CD4, HIV treatment or undetectable viral load) or among persons whose quantified result of what is being measured by the assay never crosses the critical threshold to differentiate a recent from long standing infection. To address these inaccurate results the following methods have been used. These methods are used to complement each other with the direct approach correcting results arising from clinical indicators indicative of a long-standing infection and the indirect approach correcting for long-standing infections which appear to be recent due to slower progression.

4.4.1 Direct approach

The direct approach takes into consideration patient-based information to identify longstanding infections regardless of the assay result. These data include evidence of a CD4 cell count <200 or an AIDS-defining condition at time of sample date, previous HIV positive test results or evidence that the individual is on ART or has an viral load <400 copies/ml (figure 4.2). This method helps to reclassify those with a long-standing infection and minimise, but not eliminate, the false recent rate. This direct method with the assay result is known as the Recent Infection Testing Algorithm or RITA.

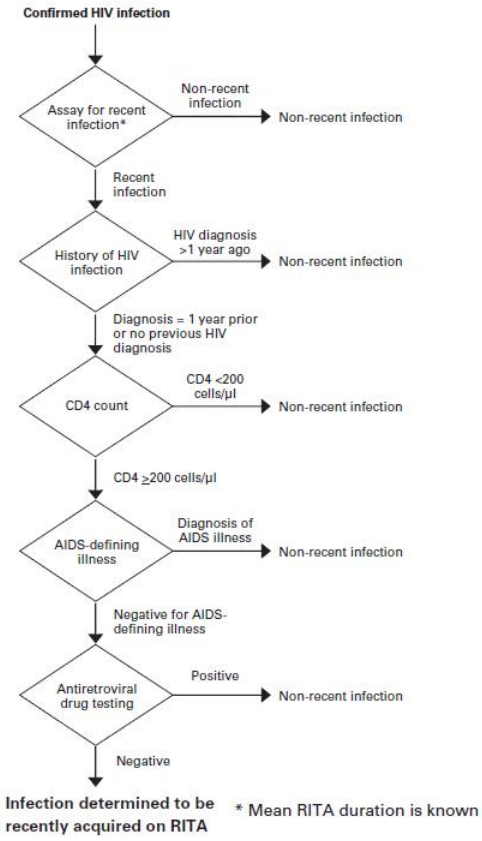


Figure 4.2: Suggested approach for minimising the risk of misclassification.

Source: World Health Organisation 2011 [405].

An alternative algorithm would include the use of more than one test for recent infection assay. This Multiassay Algorithm (MAA) has been incorporated in multiple studies with results suggesting increased accuracy [231, 343, 419, 420]. Each study used the BED-CEIA and Avidity index as part of the algorithm and suggested the process in figure 4.3, authors indicated that this method was also successful if CD4 cell counts are not available.

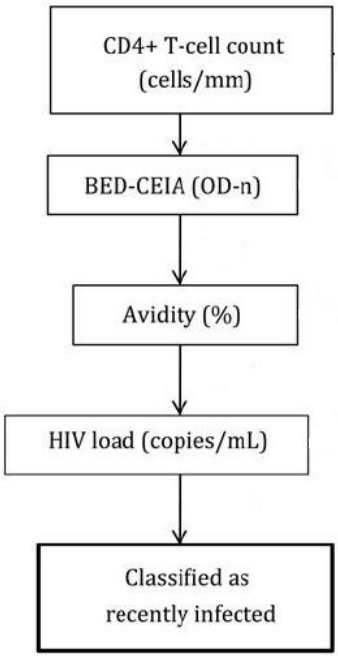


Figure 4.3: Multi-assay approach for minimising misclassification of recent infections using more than one test for recent infection.

Source: Laeyendecker *et al J Infect Dis* 2013 [343].

4.4.2 Indirect approach

The indirect approach applies a correction factor to the incidence formula derived from the fraction of persons who remain non-reactive and under the threshold for time > Sensitivity + Short-term Specificity ($>\mu_1 + >\mu_2$) or 1-long-term specificity referred to as the FRR (figure 4.4).

To determine the FRR, samples from persons known to have been infected for a time period equal to twice the recency period ($>\mu_1 + >\mu_2$) are classified using RITA, which is applied to the appropriate incidence formula. This fraction, or FRR, (samples classified as recently infected which are in fact long-standing) is removed from the number of samples classified as recently infected using RITA. Before calculating the FRR, it is necessary to ensure the same algorithm (see section 4.4.1) for identifying recent infections is also used on those known to have been infected for greater than one year.

Initially, the correction factor was estimated using the sensitivity (σ), short-term specificity (ε_1) and long-term specificity (ε_2) of the test as seen in formula 4.15 and 4.16 [202]. Using the following 3 assumptions; recent infections are randomly distributed, the window period for ε_1 is equal to the window period for σ and the window period for ε_2 is more than the window periods for σ and ε_1. To calculate σ and ε_1 samples are required from persons with a known date of seroconversion and for ε_2 samples are required from persons known to have been positive for greater than a year.

$$\frac{fN_{\mathrm{R}}}{fN_{\mathrm{R}} + \omega N_{\mathrm{N}}} \tag{4.15}$$

$$f = \frac{(\frac{N_{\mathrm{R}}}{N_{\mathrm{P}}}) - \varepsilon_2}{(\frac{N_{\mathrm{R}}}{N_{\mathrm{P}}})(\sigma + \varepsilon_1 - 2\varepsilon_2)} \tag{4.16}$$

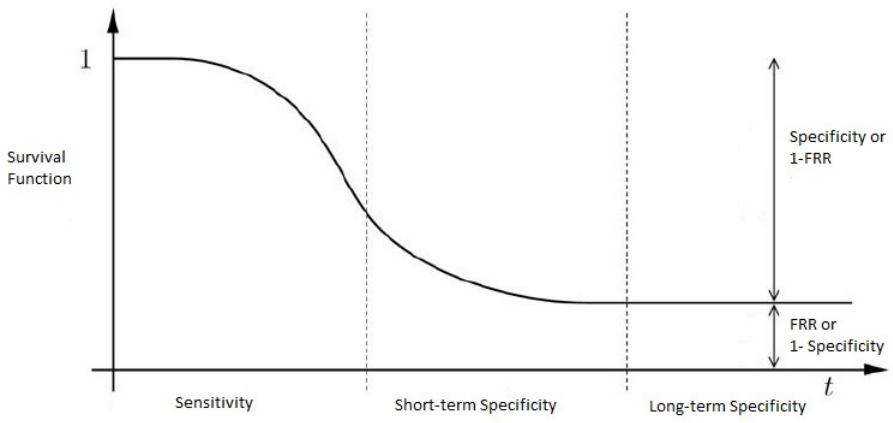


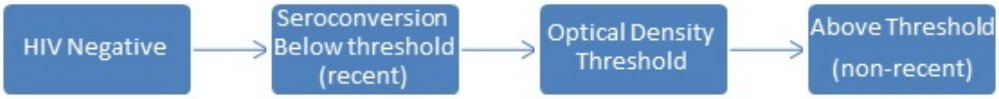
Figure 4.4: Survival curve representing the proportion of samples RITA positive by time since seroconversion.

Source: McWalter *et al PLoS ONE* 2009 [421].

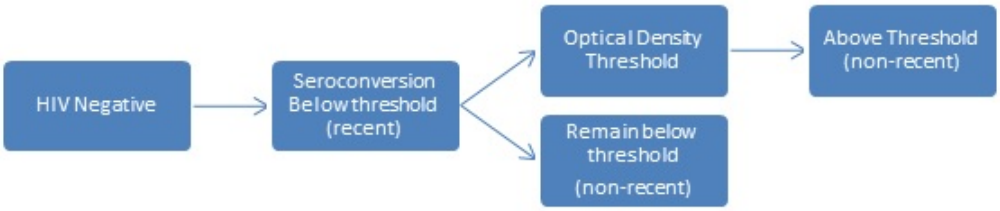
Formula 4.17 removes the correction factor, suggesting it is only necessary to calibrate 1-ε_2 or ε because $\sigma \approx \varepsilon_1$, where ε is estimated from samples known to have been positive for greater than a year [320].

$$\frac{N_{\mathrm{R}} - \varepsilon N_{\mathrm{R}}}{N_{\mathrm{R}} + \omega N - \varepsilon(N_{\mathrm{P}} + N_{\mathrm{N}})} \tag{4.17}$$

Formula 4.18 has been derived from the mathematical relationships between sensitivity, short-term specificity and long-term specificity [421–424]. McWalter and Welte indicate that not all individuals will progress over the threshold for differentiating a recent infection from non-recent, moving from figure 4.5a to 4.5b. Therefore being under the threshold and alive does not reach 0 and is flat for time >μ1 + μ2 as seen in figure 4.4, the long-term specificity. The assumptions for formula 4.18 are modifications of those previously defined by McDougal: being under the threshold and alive is flat for time >μ1 + μ2, and progressing individuals under the threshold are distributed during 0,μ1 + μ2; infection times are uniformly distributed and with the same intensity as for time μ1 + μ2 and survival is the same for progressing and non-progressing individuals [421–424].



a.



b.

Figure 4.5: Time since infection, a). Where all individuals cross the threshold from recent to non-recent b). Where a proportion of individuals remain under the threshold and therefore are misclassified as recently infected.

Source: Wang *et al JAIDS* 2009 [425].

With these assumptions McWalter and Welte [421–424] indicate that σ-ε_1+ε_2=1 or σ-ε_1=1-ε_2. As a result σ and ε_1 are no longer required, much like formula 4.17.

$$\frac{N_{\mathrm{R}}(\frac{\varepsilon}{1-\varepsilon})(N_{\mathrm{P}}-N_{\mathrm{R}})}{\omega N_{\mathrm{N}}} \tag{4.18}$$

For formula 4.17 and 4.18 the advantage of only using ε is that it is estimated through follow up intervals which are greater than $\mu 1 + \mu 2$, whereas σ and ε_1 need specimens from individuals where the time from infection can be confidently determined [320, 421–424]. What this model does not account for is false positive results arising from established infections with low antibody response, due to an AIDS defining illness or ARVs, assuming that all individuals progress in one direction from being identified as recently infected to being identified as long-standing. Further limitations considered are the assumptions that survival expectation is the same between progressing individuals and non-progressing individuals and the regional variability for the correction factors.

Formula 4.18 was found to agree with the maximum likelihood estimator [425] concluding that characterising the proportion of those individuals who remain under the threshold for time $>\mu 1 + \mu 2$ is essential [425].

Table 4.4 shows variations in incidence estimates using the different incidence formulas previously described. Data was taken from a longitudinal population-based study collected by the Africa Centre for Health and Population Studies, South Africa [125], where the HIV incidence was measured as 3.09 per 100 people per year, adjusting for sex and age. Incidence estimates which corrected for long-standing infections classified as recent were comparable to those measured longitudinally.

This correction factor was also used when estimating the true number of recent infections among new HIV diagnoses [349] as discussed in section 4.3.

$$\frac{N_{\mathrm{R}}-N\varepsilon}{1-\varepsilon} \tag{4.19}$$

Formula	N_R	N_P	N_N	σ	1-ε_1	1-$\varepsilon_2(\varepsilon)$	μ	f	ω	Annualised incidence per 100 persons at risk
$(\frac{N_r}{N_r+N_n})\times(\frac{365}{\mu})$ [117] Formula 4.2	165	2519	9236				153			4.19
$\frac{[(\frac{365}{\mu})\times N_r]}{[N_n+((\frac{365}{\mu})\times N_r)]}$ [118] Formula 4.3	165	2519	9236				153			4.09
$\frac{fN_R}{fN_R+\omega N_N}$ [202] Formula 4.15	165	2519	9236	0.768	0.277	0.0169		0.733775053	0.419178082	3.03
$\frac{N_R-\varepsilon N_R}{N_R+\omega N-\varepsilon(N_P+N_N)}$ [320] Formula 4.17	165	2519	9236			0.0169	153		0.419178082	3.19
$\frac{N_R(\frac{\varepsilon}{1-\varepsilon})(N_P-N_R)}{\omega N_N}$ [421] Formula 4.18	165	2519	9236			0.0169	153		0.419178082	3.22

Source: Barnighausen *et al PLoS ONE* 2008 [125]

Table 4.4: Incidence estimates using longitudinal population-based data collected by the Africa Centre for Health and Population Studies, South Africa.

4.5 Minimising False Long-standing Infections

Alongside minimising the risk of misclassification of long-standing as recent infections, consideration should also be given to minimising the risk of recent infections which have been classified as long-standing. Like the FRR the rate of long-standing infections which have been misclassified should also investigated, however this is much more difficult to calculate, as a population known to be recently infected would need to be identified. Other methods for reducing misclassified results include information on previous negative tests within the past 6 months, a previous documented HIV RNA or p24 positive result whilst antibody negative within the last 6 months, using clinical symptoms representative of a seroconversion or using more than one RITA assay.

4.6 Further Considerations

Using case-based reporting as the sampling framework is also subject to reporting delays, underreporting of variables, the ability to eliminate duplicate reports and the ability to identify which diagnoses are new. Furthermore, the formula used for case-based reports is used to calculate the probability of an individual testing within the recency period by either using testing history or assuming a constant rate of testing, where the date of infection is independent of the testing date. Information on testing history is limited as it may be subject to recall bias and the assumption of a constant testing rate is impacted by changes in frequency due to testing motivations such as risky behaviour or symptoms. In addition to changes in testing frequencies, are changes to testing behaviours. The introduction of specific prevention strategies or behavioural interventions may result in an increase in testing rate among those at risk. These changes impact the ability to investigate temporal trends. Finally, certain subgroups maybe underreported due to social stigma and discrimination relevant to the country estimating incidence. Underreporting of particular fields will make estimating robust estimates difficult, an underestimation of the population size will result in an overestimation of incidence.

4.7 Summary

The interrelationship between incidence, prevalence and duration of a disease enables any one of these three variables to be estimated based on information from the other two. The availability

of such information, however, is largely dependent on the sampling framework of the study, with the two main designs being cross-sectional and sero-positive. The fundamental difference between these two designs is the availability of information from the negative population. Initial work used the cross-sectional design testing of samples with an incidence assay from a purposely-recruited probability sample of the population of interest. This allowed investigators to estimate incidence based on the prevalence of recent infection among that population, and the recency period defined by the incidence assay. More recently, a sero-positive famework has been developed estimating incidence using a test for recent infection as part of routine HIV surveillance within a particular country, which is both cheaper and easier to implement.

Unlike the cross-sectional methodology, the sero-positive methodology extrapolates the true number of incident infections from those observed in persons newly-diagnosed based on the testing frequency of that population. In the sero-positive methodology published by Karon *et al* [409], the extrapolation process was based on an approximation formula to estimate the probability a person will test and be classified as recent. This formula included the likelihood the person tests close to their infection, and the likelihood they test recent by the incidence assay. However, estimating these components separately leads to an artificial upper boundary of the probability a person will test and be classified as recent. A further issue of this methodology is defining a person's minimal inter-test interval to be one year, which may not be appropriate. These limitations to Karon's [409]methodology led to the probability of testing and being classified as recent being underestimated. This in turn, resulted in the extrapolation methodology overestimating the true number of recent infections and, therefore, incidence. As a result, the method for estimating the probability of testing and being classified as recent for extrapolation was modified [346] to consider the decreasing likelihood of being a recent infection based on increasing time from infection.

Misclassification of longstanding infections as recent, arising from changes in the antibody response or slow antibody maturation, is an important limitation to consider when estimating incidence. Ignoring this limitation results in an overestimation of the number of recent infections within a population. Correcting for misclassified results includes using clinical data indicative of either a recent or longstanding infection, such as viral load and CD4 count. This is followed by a correction factor of the proportion of longstanding infections that appear recent even after

correcting for clinical data. This correction fraction, known as the false recent rate, adjusts for the fraction of non-recent infections which are misclassified as recent in incidence estimates. Failure to adjust for these results in incidence eing overestimated.

Finally, it is important to consider the impact of testing patterns when estimating incidence using the sero-positive methodology, if the inter-test interval differs by sub-population. Among populations for whom the inter-test interval is short, the probability of testing and being classified as recent will be much higher than for populations with a longer interval. Therefore, the probability of testing and being classified as recent is needed by sub-population, with shorter intervals more likely among high risk populations such as MSM.

To characterise populations presenting with a recent infection in each country, the sero-positive framework will be implemented, with a test for recent infection used on residual samples from all new diagnoses. Therefore, the sero-positive framework, which requires a number of parameters to be estimated, and to investigate testing behaviours by sub-populations. Although the issues surrounding Karon's methodology have been discussed within this chapter, I intend to use the method published by Karon *et al* [409] and the modified method by Prejean *et al* [346] to estimate the true number of recent infections within the population. By using both methodologies I will review the difference in incidence estimates and establish the impact the limitiations from the original method has on the outcome.

Chapter 5

Study Methodology

The methods I undertook for data collection are found in the following chapter. I describe how the data were collected and the processes I implemented to adapt collection techniques. I indicate what further data were required and methods of data collection.

I present how I validated and characterise the data, procedures chosen for identifying recent infections, and statistical methods involved in characterising recent infections and estimating incidence.

5.1 Introduction

Existing testing services within each country were used to identify newly diagnosed individuals. Data were collected as an extension of the current surveillance methods. Ethics approval for the study was received from the ethics committee in each country, and from University College London (UCL) research ethics committee (see appendix B). Patients seeking an HIV test did not experience any additional procedures and no extra blood was taken. Any additional data introduced were dependent on the data already collected by each surveillance system. Table 5.1 lists the data required and availability for each country before starting this work.

Laboratory-based methods to differentiate recent from long-standing infections using the maturing antibody response to the HIV infection were introduced and conducted on samples confirmed as newly diagnosed. Results from Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) [Gary Murphy, personal communication, March, 2013], initiated to provide technical guidance and validate the different RITA assays, were used to identify an appropriate assay.

All additional testing was conducted once all samples had been confirmed as HIV-positive. All persons tested for HIV country-wide in Estonia and persons attending one of the 30 VCT facilities in Poland between January 2013 and January 2014 were included and persons attending for an HIV test at any one of the four infectious disease clinics known as the AIDS centres in Kiev between April 2013 and March 2014. Patient results and collected data were matched using a unique patient identification number which was used throughout and, where possible, year of birth and sex. Data for Estonia and Ukraine were available throughout the study, whereas data for Poland would not be available until September 2014.

Data Group	Data Requirements	Ukraine	Poland	Estonia
Site information	Site of test	✓	✓	✓
	Identification number	✓	✓	✓
Socio-demographic data	Probable route of infection		✓	✓
	Age/Year of birth	✓	✓	✓
	Sex	✓	✓	✓
	Area of residence	✓	✓	✓
Testing frequencies and motivation	Number of negative tests within the last 2 years			
	Date of last negative test		✓	
	Date of first positive test		✓	
	Reason for test		✓	
Testing information	Date of diagnosis	✓	✓	✓
	RITA assay result			
Additional information	Clinical Stage			✓
	CD4 cell count / Date of CD4			✓
	Date of last negative test		✓	
	Date of first positive test		✓	
	Evidence of treatment			✓
	False Recent Rate			

Table 5.1: Data requirements, indicating whether these requirements are met by each country.

5.2 Ethics Statement

The study was part of CASCADE within EuroCoord (www.EuroCoord.net) funded by the European Union Framework Programme VII. Ethics approval was given by the University College London, Institute of Epidemiology Kiev, The University of Tartu, Estonia and the National Institute of Public Health National Institute of Hygiene, Poland (appendix B).

5.3 Project logistics

During the three years of my PhD, I was the project coordinator with the task of setting up the study to characterise recently acquired HIV infections within Estonia, Poland and Ukraine. As part of this project I worked with partners to ensure project ownership within each country. Collaborators involved were Ruslan Malyuta for Ukraine, Magdalena Rosinska and Janusz Janiec for Poland, and Irja Lutsar and Pilleriin Soodla for Estonia.

I was involved in all aspects of the project which included but was not limited to; overall organisation and administration of the project, writing documentation for the project and ethics committees applications, design and implementation of a new system in Ukraine, sample management, data extraction and manipulation, statistical analysis and incidence estimation.

Figure 5.1 and 5.2, shows how the project developed over the three years. In brief, during the first year, I finalised the project protocol and gained ethics committee approval from UCL for the overall project and within each country separately. I established the parameters for estimating incidence and worked with each team to identify what data already existed and what was additionally required, which included developing a new system for Ukraine.

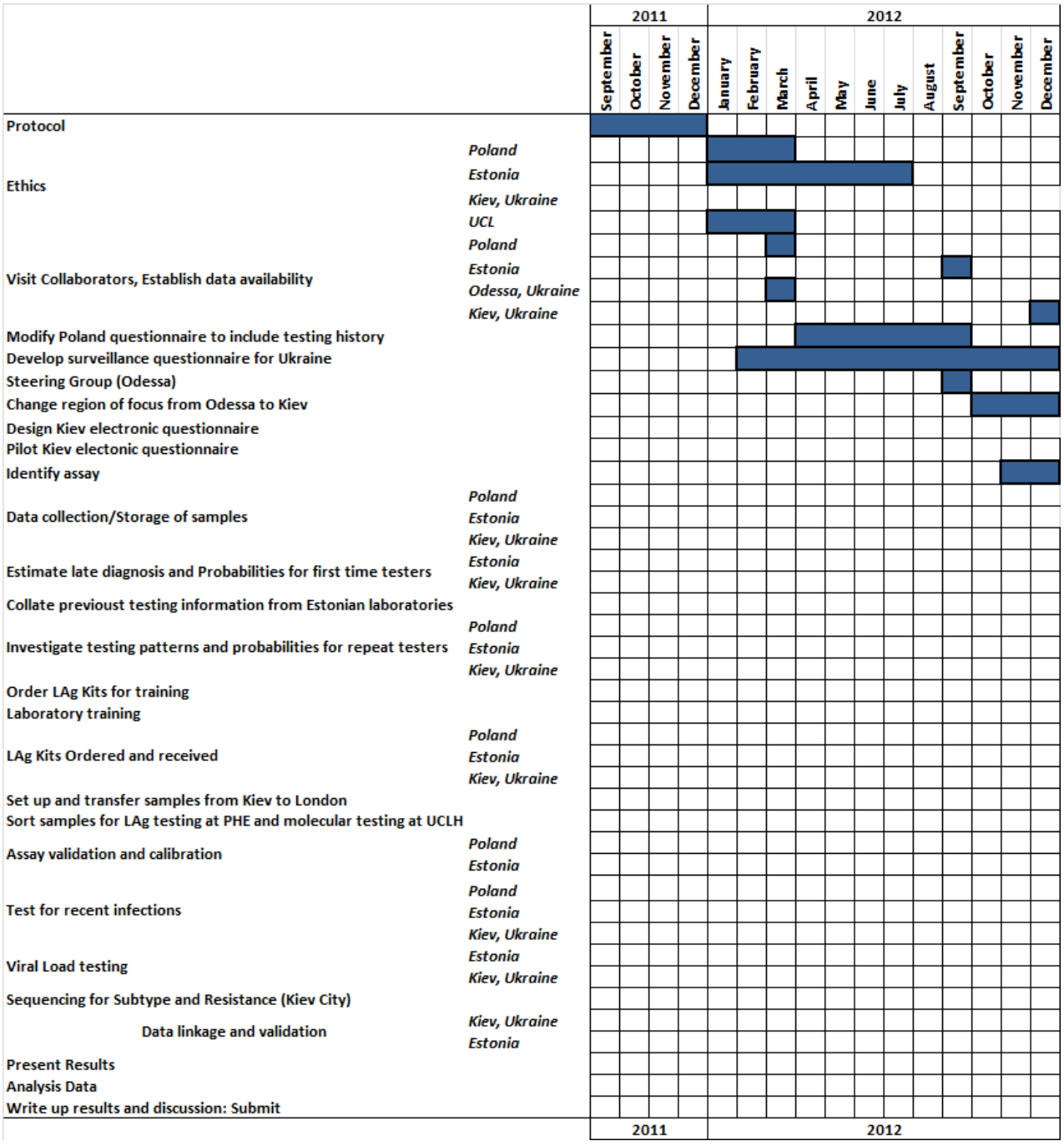


Figure 5.1: Gantt chart of the data processes within the project from September 2011 - December 2012.

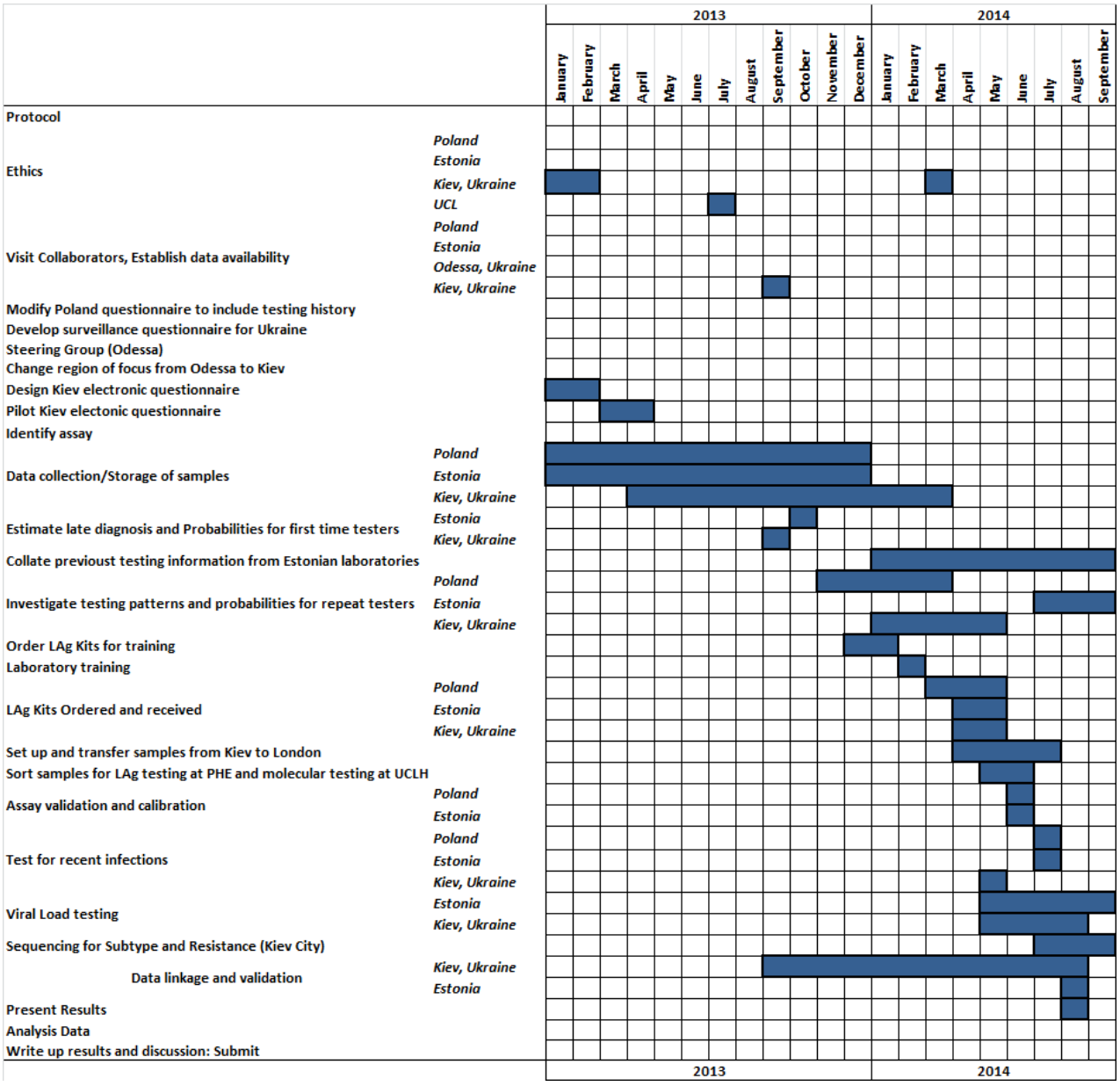


Figure 5.2: Gantt chart of the data processes within the project from January 2013 - September 2014.

Data collection and storage of residual samples took place in 2013 for both Poland and Estonia, and during the first quarter of the same period I developed and piloted the new system for Kiev. This involved developing the data collection form for use on a handheld tablet, which went live in April. Data collection and storage of samples for Kiev took place from April 2013 and throughout 2014. Although the project was overseen in the specific countries by the collaborators, I was able to review the data for Kiev monthly downloading the data directly allowing for feedback to the clinics. I also visited the testing facilities including the laboratories during the summer of 2013.

In October 2013 I was invited to attend the WHO incidence working group meeting in Rome, where I initiated a collaboration with CDC, to conduct the training of the LAg avidity EIA the chosen assay. The training took place in February 2014, where CDC and a laboratory technician from each country spent a week within a laboratory at Public Health England in London. During this time it was agreed that UCLH would conduct all the molecular epidemiology for samples from newly diagnosed persons in Kiev. Because of unrest in Ukraine, all samples including those to be tested with the LAg avidity EIA were shipped to the UK.

Data for Poland and Estonia were collated by the collaborators, whereas I worked on the data collected for Kiev City throughout, cleaning and validating the data. I match the demographical data to the test results, RITA results, and viral load measurements, and determined the individual subtype and resistance mutations using the sequences received from UCLH. Probabilities for repeat testers were estimated following data collection in 2014, whereas for first-time testers late diagnosis estimates for probabilities were collated from Estonia and Ukraine during 2013. Testing patterns were investigated for Kiev and Estonia using data collected during the project, and from previous years for Poland. The origins of data used are seen in table 5.2.

Country	Data	Source	Year
Poland			
	New Diagnoses	VCT Sites	2013
	Repeat Testers	VCT Sites	2008-2010
Estonia			
	New Diagnoses	All Testing Facilities Sites	2013
	Repeat Testers	Laboratories	2013
	Late Diagnoses	HIV Database	2010-2012
	False Recent Rate	HIV Database	
Kiev, Ukraine			
	New Diagnoses	AIDS Centre	2013-2014
	Repeat Testers	AIDS Centre	2013-2014
	Late Diagnoses	Patient Notes (AIDS Centre)	Q1: 2013
	False Recent Rate	AIDS Centre	

Table 5.2: Data Sources for each variable and country.

5.4 Data Collection

5.4.1 Ukraine

Existing country-wide testing services were used to identify newly diagnosed individuals. At the time of an individual's initial HIV test in the southern region of Odessa, the following information was collected, together with a serum sample: patient's name or clinic number, year of birth, area of residence, and reason for test (appendix C). Further demographic and clinical information, including HIV risk factors, are available on individuals who attend for care at an AIDS centre. However, it is estimated that 20% of positive individuals would be lost to follow up and no extra information would be available for them.

Working with the non-governmental organisation Perinatal Prevention of AIDS Initiative (PPAI), I worked to modify current data collection methods to introduce data needed to adequately characterise the newly-infected population and to estimate incidence. I designed a detailed anonymous questionnaire to be completed at the initial consultation (appendix C), which included all the necessary fields. Demographic information to characterise the population into sub-populations, test history to correctly classify results and testing history and reason for testing for determining the method of estimating incidence and understanding testing motivations and frequencies.

Data were collected directly from the patient, and risk factor information was collected by asking patients whether they have ever participated in activities known to be risk factors for HIV infection, rather than using labels. For example "have you ever had sex with another man?" rather than "are you a homosexual man?"

The anonymous questionnaire was piloted in five facilities during July and August of 2012 to assess acceptability from both medical workers and individuals attending for an HIV test. The completion of fields varied, demographic information (year of birth, sex, residence and citizenship) and reason for test were completed for ≥96% of individuals. Information of whether the individual had been tested previously was 93% complete when completed by the health care worker and 98% complete when completed by the individual. However, the date of previous test was only recorded by the patient. Of the 81 individuals who answered "do you think you have

been at risk of HIV?" only 7 answered "yes", although 29 individuals ticked one or more of the risk factors listed. No information was recorded on whether the individual had any evidence of symptoms.

Following the pilot, further modifications were made to the anonymous questionnaire. The final data flow is outlined in figure 5.3, with the anonymous questionnaire introduced during the patient's initial consultation. No identifiable information would be collected on the questionnaire and the clinic code would be used to link the reference list, blood sample and anonymous questionnaire when entered at the laboratory. No additional processes were added, with the anonymous questionnaire and RITA results entered onto the sample database. Remaining sera from individuals confirmed as new diagnoses would be stored and available for RITA testing.

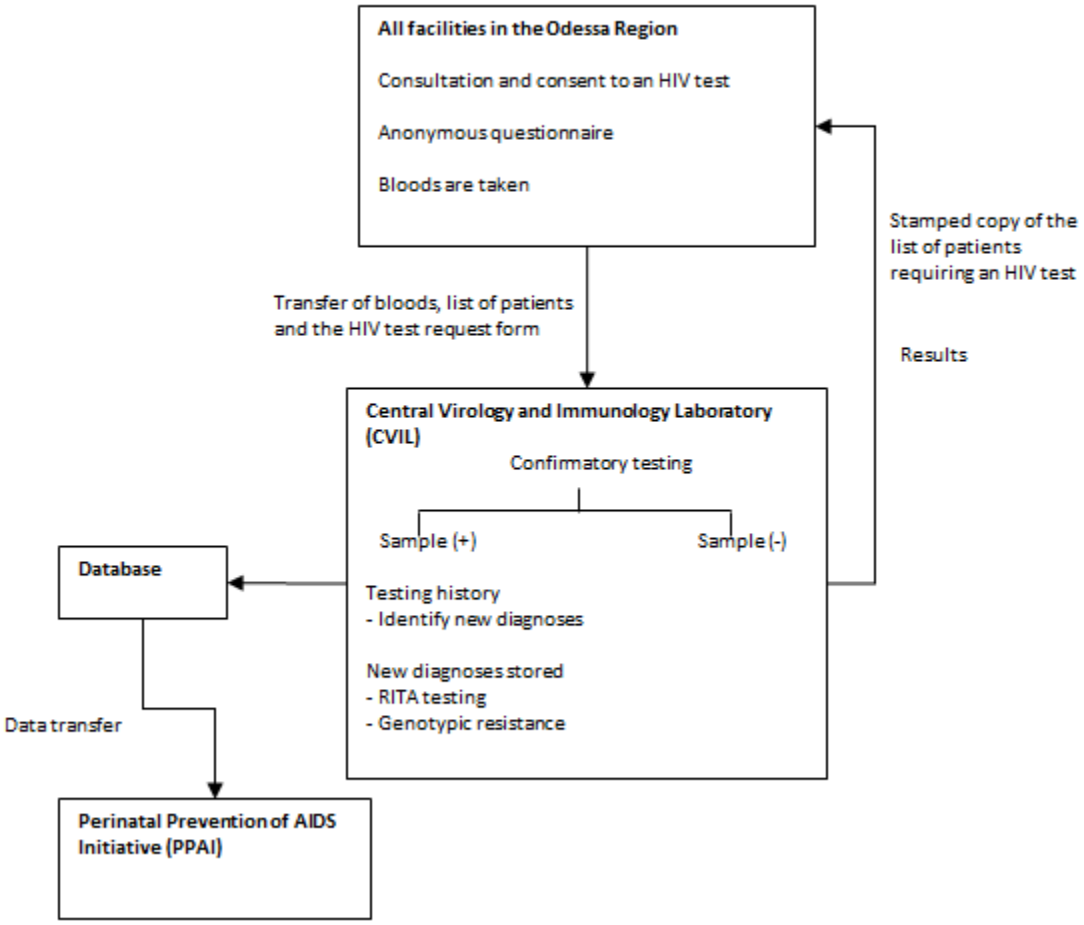


Figure 5.3: Data flow between testing site, laboratory and the Perinatal Prevention of AIDS Initiative (PPAI) in Odessa, Ukraine.

Following the Ukraine parliamentary election in October 2012, the Central Virology and Immunology Laboratory (CVIL) was dissolved. As a result, the project was moved to the Ukraine capital Kiev, where data processes and information collected at initial consultation were similar to those of Odessa. Persons attending at any one of the four Kiev City AIDS centres were the focus of the study (figure 5.4) with the same anonymous questionnaire introduced at initial consultation.

However, unlike Odessa, the patient completed the questionnaire electronically using a handheld device. A review of five different methods of data collection identified that tablet computers closely resembled the use of paper for data collection, whilst gaining the benefit of computer-based approaches [426]. A study comparing paper with tablet computers found that patients were positive about using the tablet, concluding that electronic tablets were a valid methodology for collecting data [427]. Furthermore, the use of the electronic tablet ensured that patients maintain anonymity regards sharing information on their risk factors.

All electronic questionnaires were managed by me at the Medical Research council (MRC). Medical staff at the testing centre did not have access to patients' answers to the questionnaires. The patient's sample and test request form followed the normal process with the institute of microbiology, Kiev AIDS Centre storing all positive samples for RITA testing. The modified data flow can be seen in figure 5.5, with a timeline of processes in figure 5.6.

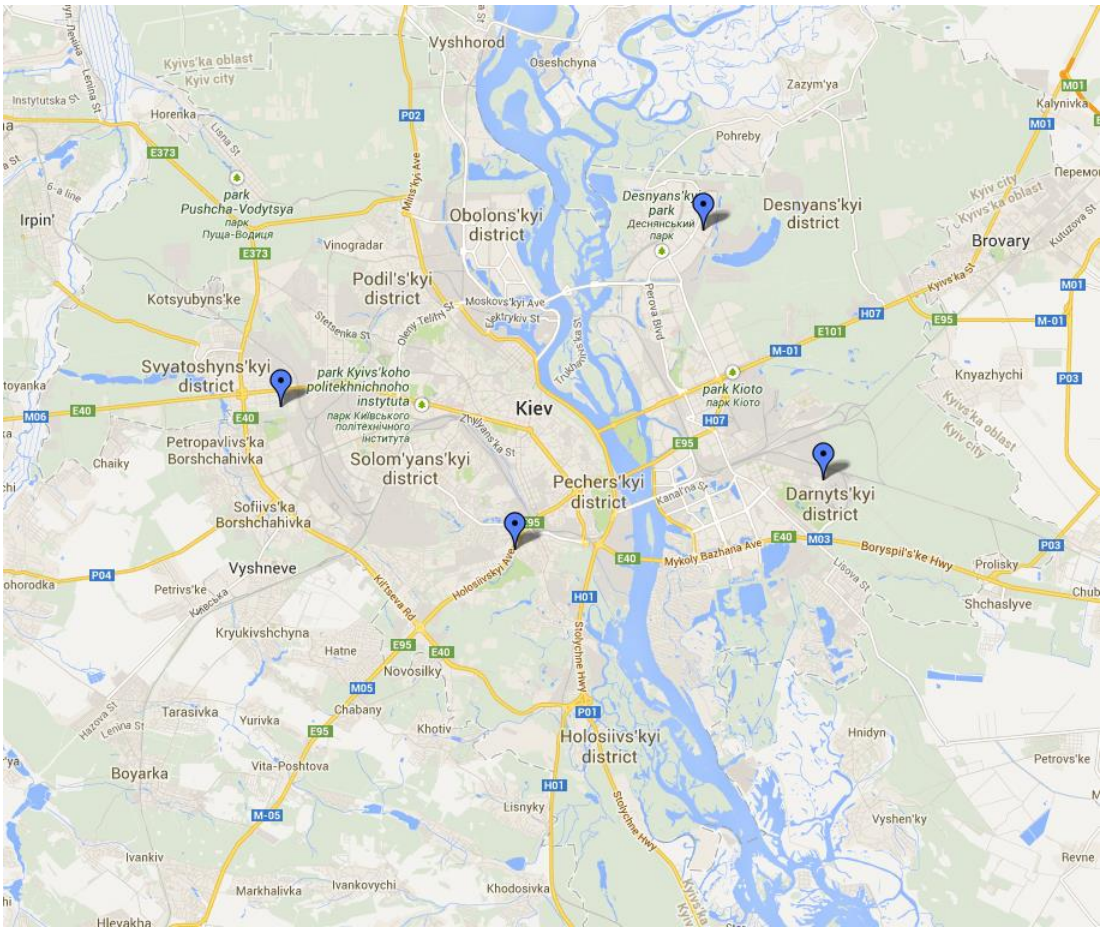


Figure 5.4: Testing sites in Kiev city oblast.

Source: Googlemaps 2013.

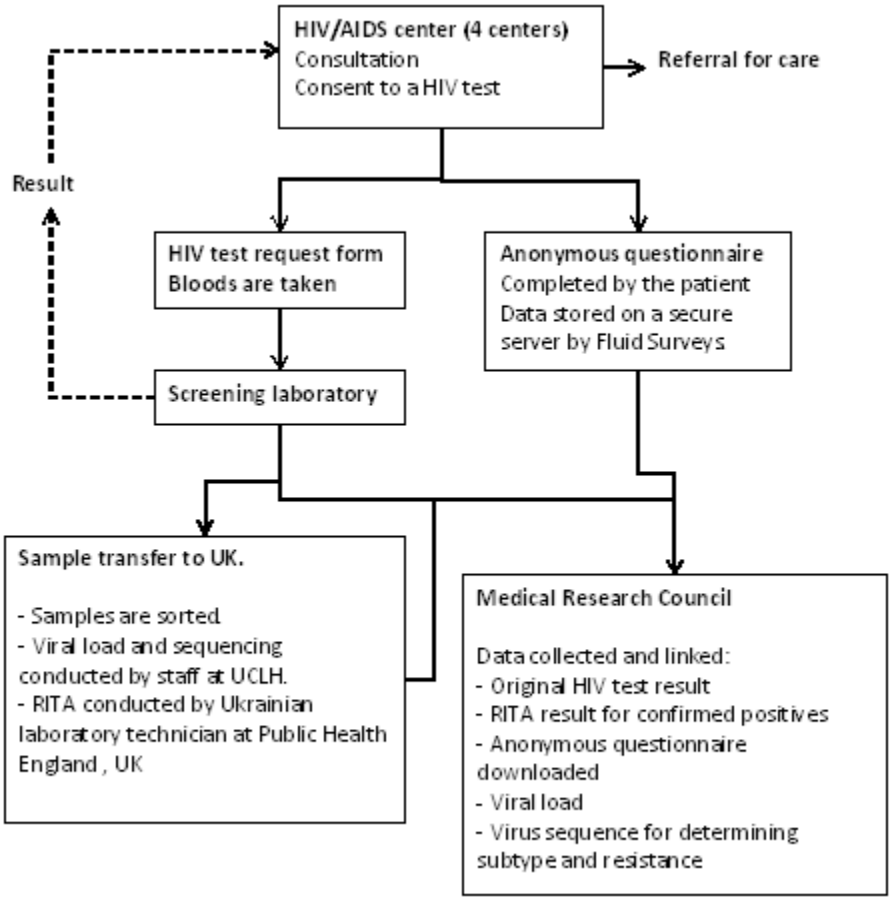


Figure 5.5: Data flow for Kiev City, Ukraine.

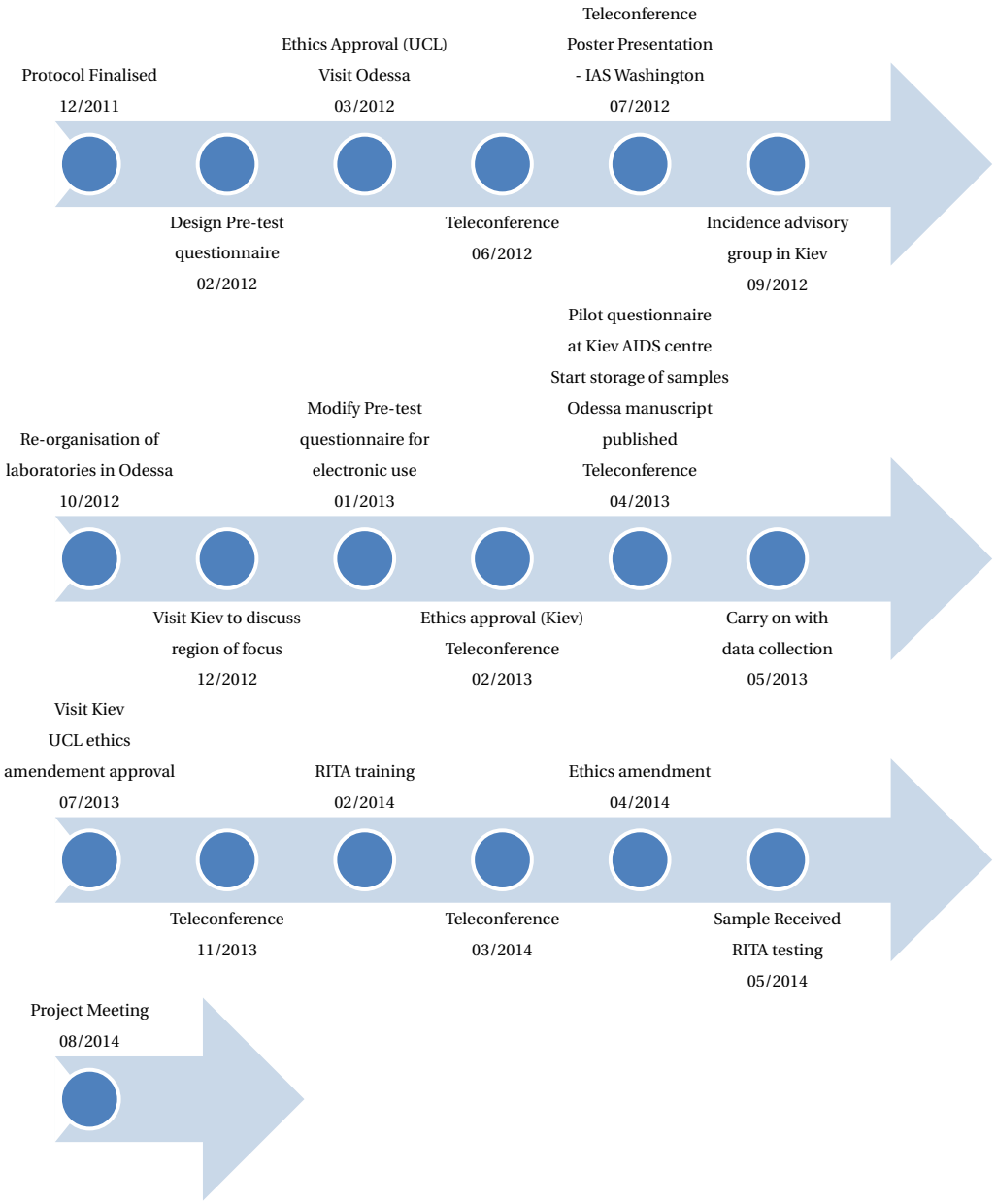


Figure 5.6: Flow of processes and communication within Ukraine.

Preliminary work to classify recent infections in Odessa were conducted before this study on all new HIV diagnosis between May and December 2009. The BED-CEIA assay was used where adults with a standard optical density in a confirmatory test of ≤ 0.8 were classified as recent and this >0.8 as long-standing [396]. I analysed there data as part of this thesis.

5.4.2 Poland

Persons attending one of the 30 VCT facilities for an HIV test were used to identify newly diagnosed individuals (figure 5.8). At initial consultation information is collected from the patient through a detailed pre-test questionnaire and self-reported questionnaire, alongside a test referral form which is sent with the patient's blood sample to the laboratory. Variables collected using the pre-test and self-reported questionnaires are listed within appendix C. The pre-test and self-reported questionnaires are forwarded to the national AIDS centre for data entry and all information is collated at the National Institute of Public Health - National Institute of Hygiene (NIZP-PZH). Paper forms are forwarded to the national AIDS centre at the end of each year to be processed and data are only accessible in September of the following year.

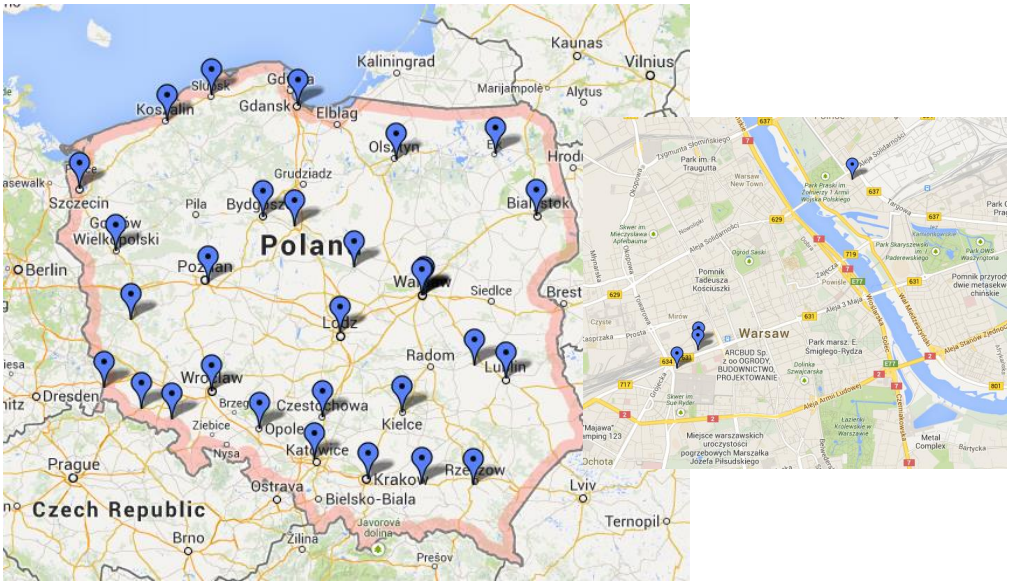


Figure 5.8: Distribution of VCT sites in Poland.

Source: Googlemaps 2013.

The patient's blood sample and test referral form (appendix C) are sent to screening laboratories and those identified as positive are sent to one of two confirmatory laboratories. Variables collected on the test referral form include initials or password, year of birth, sex, nationality, and probable route of infection. Test results are sent back to the testing facilities and surveillance laboratory reports are available at NIZP-PZH within 30-90 days.

Patients with a confirmed positive result can attend for HIV-related care at one of 15 specialised clinics in each of the major cities in Poland. Further specialised test are offered including CD4 and viral load counts and access to ARVs.

Working with NIZP-PZH, I identified that no modifications to how patient information is collected is required. However, the question "Number of negative tests within the previous two years" was added to the pre-test questionnaire. All data and results will be collated and matched by staff at NIZP-PZH. The final data flow and project timeline can be seen in figure 5.9 and figure 5.10.

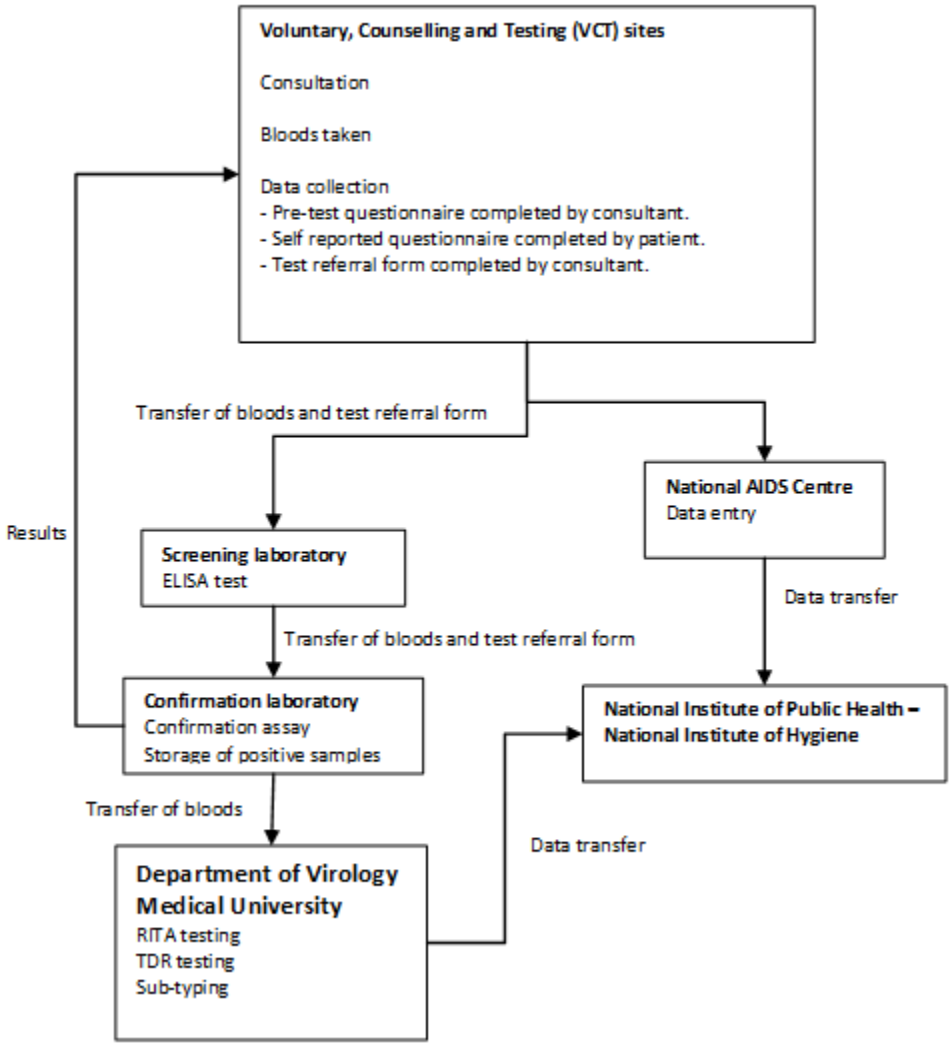


Figure 5.9: Data flow between testing site, laboratory and the National Institute of Public Health-National Institute of Hygiene.

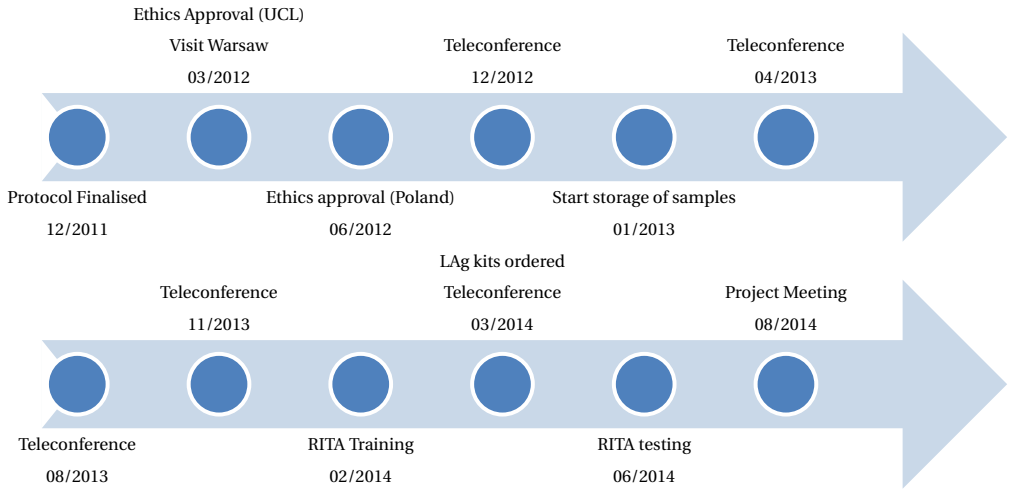


Figure 5.10: Flow of processes and communication within Poland.

5.4.3 Estonia

Existing country-wide testing services were used to identify newly diagnosed individuals. Following laboratory confirmation, patient's results are sent back to the testing facility and patient's with a confirmed HIV positive result are referred to one of five hospitals offering HIV-related care; these include Tartu University (SATUK), West-Tallinn Central Hospital (LTKH), Narva Hospital, IDA-VIRU Central Hospital (IVKH) and East-Tallinn Central Hospitall (ITKH). At these centres, information on the patient is collected through the NAKIS HIV report linked to the Estonian health board and the HIV database, both forms of data collection are web-based. Before information is entered onto the HIV database informed consent is required from the patient. Variables collected using the NAKIS HIV report form and the HIV database are listed within the appendix C.

All Estonian citizens have a unique ID code which is recorded for all confirmatory tests and linked to the patient's unique HIV code. Double registration of the unique ID code is not allowed. All results and information collected on each patient is linked to the HIV code. The link between ID and HIV code is kept confidential.

Working with the Institute of Microbiology, I identified that no modifications to how patients' information is collected is required. However, information on previous testing history were collated from confirmatory laboratories. The data flow and timeline are outlined in figure 5.11 and figure 5.12.

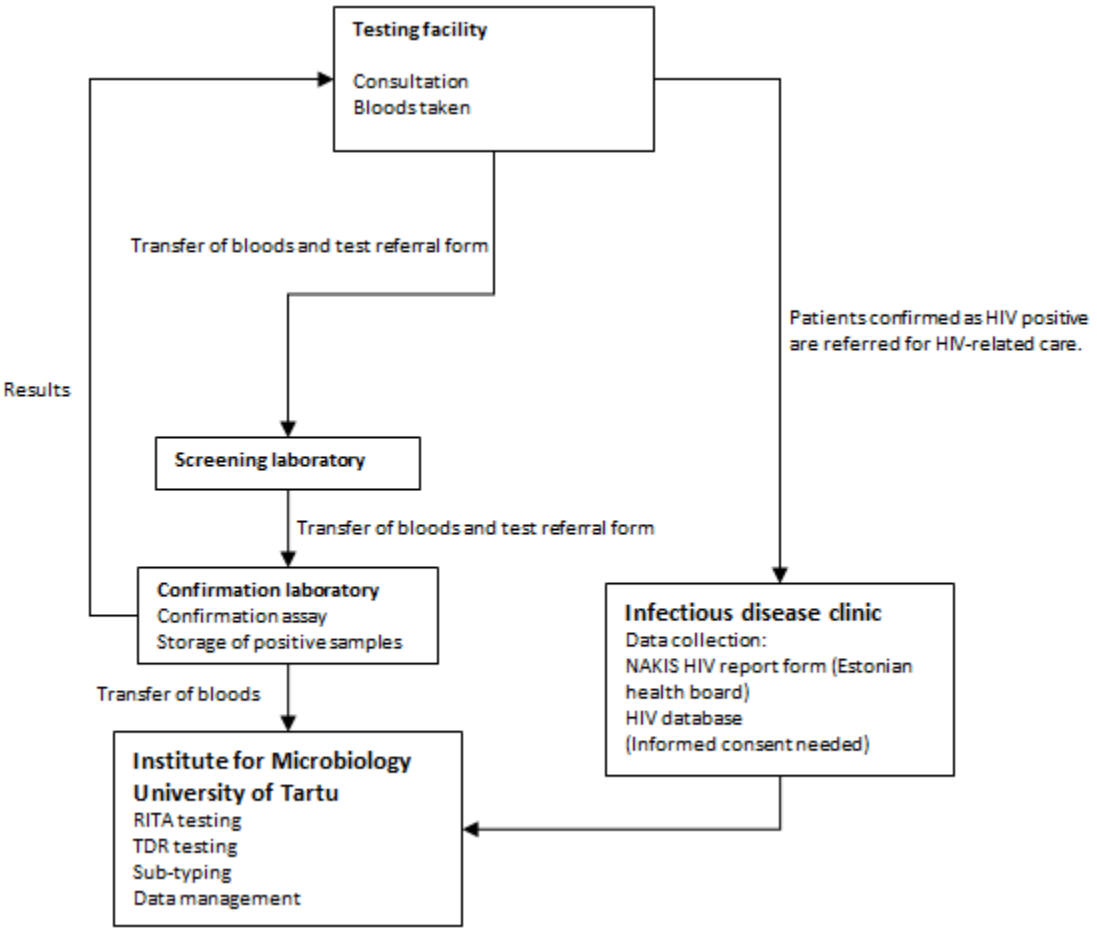


Figure 5.11: Data flow between testing site, laboratory and infectious disease clinic.

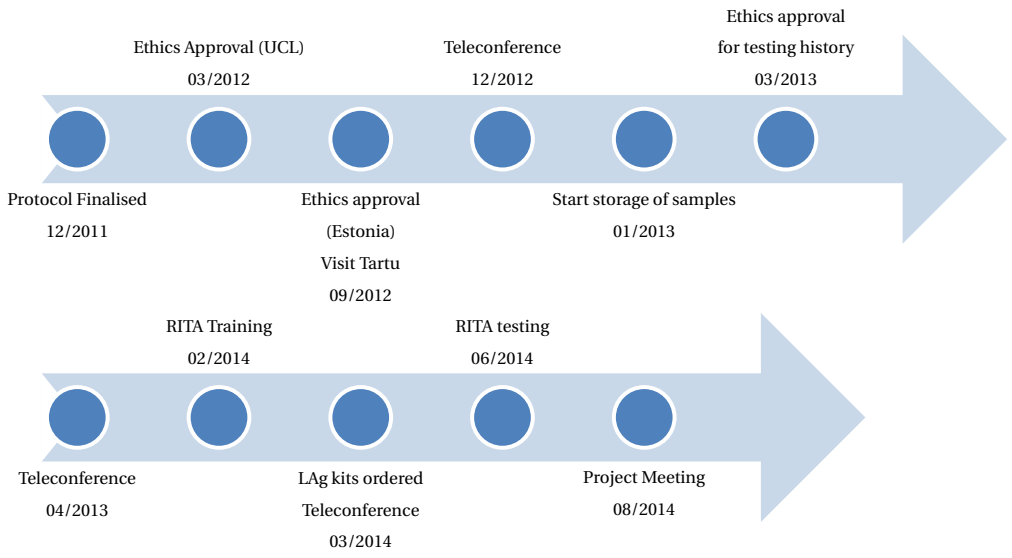


Figure 5.12: Flow of processes and communication within Estonia.

5.5 Data Validation and Characterisation

Data cleaning and validations were completed by the data management team initially in Poland and Estonia. I conducted further validations to categorise probable route of exposure, reason for test and testing history, detailed below. For Kiev, I conducted all cleaning and validations, initially de-duplicating records using year of birth, sex, clinic and clinic identification number and removing any incomplete records, where year of birth, sex or date of diagnosis were incomplete. Information from duplicate records were merged, where information differed records were sent back to the laboratory and records were merged based on the results. From each dataset, records were removed where the previous positive result was reported as positive, as these persons cannot be classified as newly diagnosed.

Data are presented for adults, persons aged 16 years and over for Ukraine and Poland and 18 years and over for Estonia.

Probable route of exposure was reported directly for Kiev City and Estonia, whereas for Poland probable route of exposure was assigned using data collected through the pre-test questionnaire. PWID was assigned where risk was identified as injecting drug use, person reported sex under the influence of drugs by injection and/or sharing a syringe. MSM was assigned if the sexual orientation was homosexual, and for males reporting male sexual contacts, a male partner ever, passive anal sex and/or reporting a homosexual partner. Heterosexual contact included reporting heterosexual contact, reported vaginal sex, having the opposite sex as a sexual partner and/or reporting a heterosexual partner. Other risks were reviewed and where no information was available person's risk was classified as not known. For each country, probable route of exposure was allocated according to a hierarchical order, where persons with more than one reported risk were classified by the risk most likely (PWID, MSM followed by heterosexual contact).

Reason for test was grouped into the following four categories: clinical indication (persons presenting with symptoms), high risk population (those regarded to have high risk behaviour including injecting drugs, contact of a known positive, diagnosed with a sexually transmitted infection, individuals who experienced an occupational HIV risk and sex with multiple partners) and general screening (blood, organ and tissue donors, before surgical interventions, women

screened through antenatal services, recruitment in to the army, prisoners and persons requiring HIV due to regulations or policy, e.g. for employment or visa procurement).

For the data collected through the previous study in Odessa, information was available on: basic demographic information such as name, year of birth, residence, and reason for test. No information was available on previous AIDS diagnosis or prior use of ART. However, HIV positive individuals were referred to a clinical centre for HIV/AIDS, available in each province, where HIV care is provided. Further information on HIV disease stage and whether persons had previously been on ART was available for individuals who took up the referral.

I grouped reason for test into the following four categories: clinical indication (individuals presenting with an illness suspected to be due to HIV infection, and those who had died and an autopsy revealed HIV infection), high risk population (those regarded to have high risk behaviour including sex with a prostitute and "multiple partners", diagnosed with a sexually-transmitted infection, and individuals who experienced an occupational HIV risk), general public screening (blood, organ and tissue donors, women screened through antenatal services, recruitment into the army, prisoners, and persons requiring HIV testing due to regulations or policy, e.g. for employment or visa procurement), and other (foreign citizens and anonymous testing).

5.6 Defining Recent and Non-Recent Infections.

A recent HIV infection was identified using the SediaTM HIV-1 LAg Avidity EIA, an in vitro quantitative Limiting Antigen (LAg) avidity enzyme immunoassay [389, 390, 428]. The LAg avidity EIA is a CDC developed assay which uses the avidity index, the strength of the bond between the viral protein (antigen) and the HIV-specific antibody (section 3.2.5), to differentiate recent from longstanding infections (section 3.2.5). A high concentration of antigen results in binding from both high- and low- avidity antigens, by decreasing the concentration of the antigen coated on the well, means that only the high avidity antibodies ,the long-standing infections, are able to bind. The avidity result is measured by the concentration of the bound antibodies [390], with a low avidity representing a recent infection, whereas a long-standing infection would have a high avidity. Using the procedure described in figure 5.13, an optical density is measured with those measuring below 1.5 being classified as "recent".

The LAg Avidity EIA was performed according to manufacturer's instructions (Sedia Biosciences Corportaion, Portland, Oregon) [429], shown in figure 5.13. The normalised optical density is estimated by dividing the optical density of the specimen by the mean optical density of the calibrator. A recent infection is equal to an optical density of <1.5 and a mean duration of recent infection of 130 days (95% CI: 118-142) [429]. Initial testing requires all samples to be tested once, and for samples with an ODn ≤ 2.0 to be tested in triplicate for confirmation. As the LAg Avidity EIA uses well plates no specialised machinery is required.

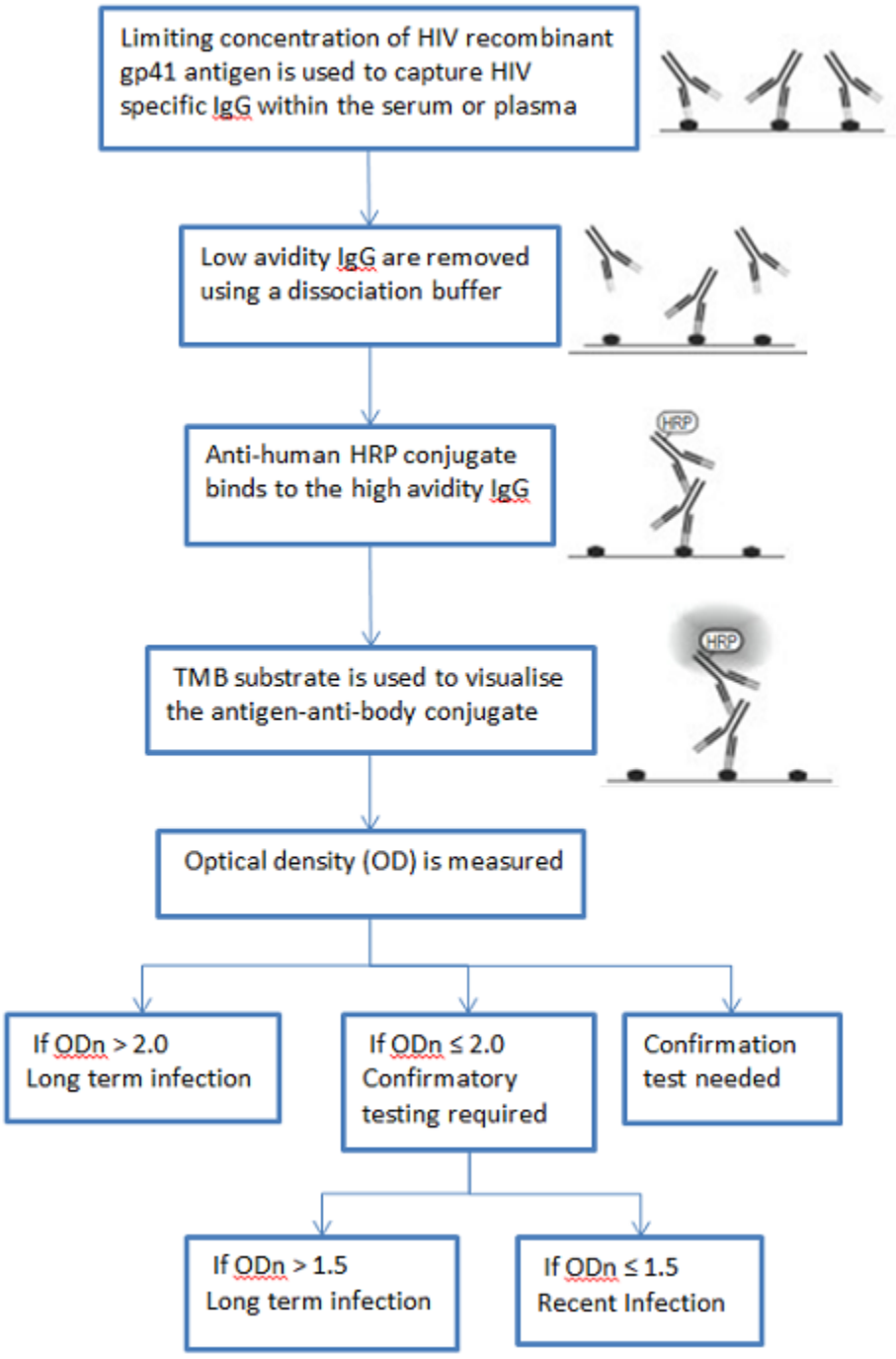


Figure 5.13: LAg-Avidity EIA procedures.

Source: Sedia HIV-1 LAg-Avidity EIA insert [428].

The LAg Avidity EIA was one of the TRI being evaluated by CEPHIA, residual samples from persons with particular clinical characters were used in a qualification panel to estimate the MDRI, FRR, the coefficient of variation (CoV) and the reproducibility. When evaluating the performance of the LAg, CEPHIA identified that LAg produced the lowest FRR of 0.9% (95CI: 0%-4.7%), however like all of the TRI evaluated the LAg performed poorly on specimens with a low or undetectable viral load and/or currently on treatment [Gary Murphy, personal communication, March, 2013]. Therefore a viral load was required for all samples with an OD <1.5, where a viral load <1000 was classified as longstanding (figure 5.14).

Samples collected in Kiev City were transported to the UK for testing with the LAg Avidity EIA conducted at Public Health England and Viral loads at the University College London Hospital. For both Poland and Estonia, all testing were conducted in the corresponding country.

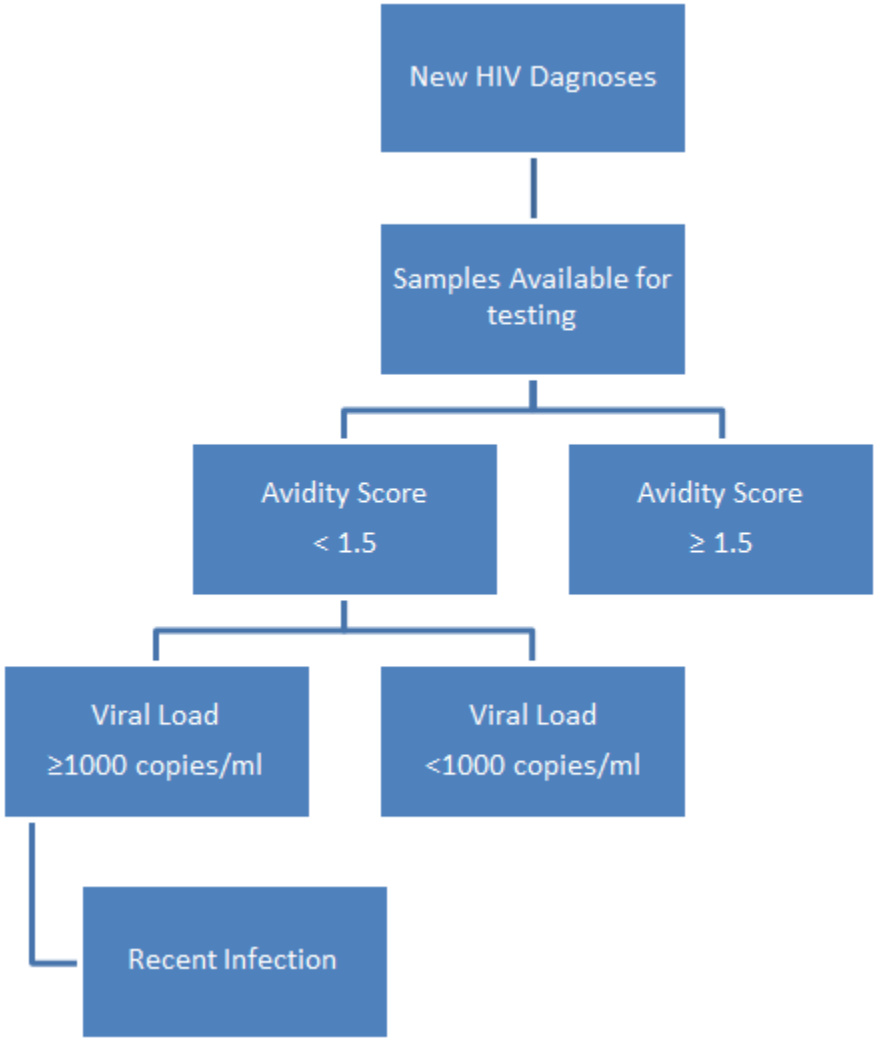


Figure 5.14: Recent Infection Testing Algorithm (RITA).

5.7 Molecular Epidemiology

The circulting HIV subtype and prevalence of transmitted drug resistance (TDR) among persons classified as recently infected were ascertained using residual samples from persons presenting for an HIV test. Samples were collected as described in section 5.4.

For Poland and Estonia testing was conducted in the respective laboratories conducting the test for recent infection, for Kiev, Ukraine samples were transferred for testing at the University College London Hospital (UCLH). The definitiion of drug resistance mutuations was taken from a standardised list used for genotypic surveillance of TDR [430]. Specific mutations were identified from sequences, as was viral load (copies/ml) and HIV subtype.

5.8 Incidence Estimation

I describe the newly-diagnosed population overall and by sub-populations including age at diagnosis, sex, reason for testing and risk factors. I further describe those identified as recently infected overall and by sub-populations, exploring the association between testing recently and key variables collected at the patient's initial consultation using logistic regression models.

HIV incidence was estimated using the stratified extrapolation method published by Karon *et al*, and later modified by Prejean *et al*, described in Section 4.3. Briefly this method uses the observed number of recent infections and the probability that a person will present for a test and have a recent infection to estimate the true number of recent infections in the study period. The probability of testing and being classified as recent, is the product of the probability of testing within a year of infection (P_1) and the probability the result is recent within a year of infection (P_w). P_1 is estimated separately for persons who have tested previously and for persons where this test is their first. For persons with testing history the probability of testing within a year is estimated as the time between the persons last negative and first positive test, whereas among persons testing for the first time the time between infection and diagnosis is estimated using the proportion of persons who do not test until late within their infection, defined as a CD4 cell count <200 and/or and AIDS diagnosis within 3 months of initial diagnosis. P_w, the probability that a test will be classified as recent is equal to the annualised mean recency period, dividing the reference period (365 days) by the MDRI (130 days), which results in a probability of 0.356.

For Kiev and Poland testing history was self-reported by the patient and in Estonia information on testing history within the previous two years will be collected from laboratories. Persons were classified as a repeat tester if there was evidence of at least one previous HIV test. Previous test was coded as not known if no date or result of the previous test was reported, or the previous test result was equivocal and where the previous test was within a month of the current test. For Estonia and Kiev city data on previous test was collated from the population of interest, however, for Poland, I used data from persons testing at a VCT between January 2008 and December 2010. The data for each year are collated separately and cannot be de-duplicated because data is collected anonymously; therefore persons with a previous test occurring during the three year period and conducted at a VCT clinic were also excluded.

The proportion of persons diagnosed late was not derived directly from the study population. Data on patients' clinical status and CD4 cell count were collected from patient notes during the first quarter 2013 in Kiev city, and for Estonia these data are available on the HIV database for patients who take up a referral for care. Data from reports of newly diagnosed HIV and AIDS cases reported to the provincial sanitary-epidemiological stations were readily available for Poland, whereas data on CD4 cell counts were stored by the HIV clinics. Late diagnosis was defined as an AIDS diagnosis and/or a CD4 cell count <200 within 3 months of initial HIV diagnosis.

Differences in testing frequencies among sub-populations were also investigated, to determine whether probabilities for testing within a year would differ by these subpopulations. For each population using the data collected of testing history I explored the association between a repeat test and key variables collected at the patient's initial consultation using logistic regression models.

Because there were missing data I used a multiple imputation method. The STATA program MI (Multiple Imputation) was used, which is a simulation statistical technique where 20 imputations were generated using a logistic regression imputation model. Using the variables ID, diagnosis date, sex and age, which were complete for each individual the imputation model was performed separately for each imputation and later pooled into a single multiple-imputation result from

which the missing value was replaced. Variables which were imputed were residence, HIV test result, probable route of exposure, Reason for test, previous testing history and evidence of a recent infection.

Confidence intervals were estimated using bootstrapping methodologies where the original data were resampled 1000 times to create 1000 datasets from which the probabilities for testing and being classified as recent were estimated to give the upper and lower estimates where 95% of the time the point estimate will fall within these limits.

Rates per 100,000 population were calculated for tests, diagnoses and incidence estimates. Population denominators were based on 2012 data published by the statistical office of the European Union, Eurostat [431]. Region specific denominators for Kiev City were based on data collected through the government census [432]. Rates were calculated by dividing the number of tests, diagnoses or estimated number of recent infection for the calendar year by the population denominator and multiplied by 100,000. Incidence rates per year will be calculated for each country overall and by age and sex using population estimates as the denominator. Where available population estimates on subpopulations at risk of HIV were collated, however for hard to reach and stigmatised populations such as PWID and MSM in Eastern Europe these data are unlikely to be available.

For Ukraine, the AIDS Alliance [433], published population estimates for most at risk populations (MARPs) by region. Estimates for 2012 indicate that in Kiev City there were an estimated 41,500 MARPs, of which 23,400 were PWID and 9,400 Men who have sex with men. These figures are estimated using the method of 'anonymous friend', where persons are asked to list the behaviours of friends, data from behavioural studies conducted among at risk populations and sociological surveys of young people aged 14-24 years and of adults aged 25-49 years, further details are within the analytical report based on sociological study results Estimation of the Size of Populations Most-at-Risk for HIV Infection in Ukraine in 2009 [434]. MSM population estimates derived from the European MSM internet survey (EMIS) were also used [435], which considered the diagnosed population for a country, survey members and the diagnosed survey members. The proportion estimated to be MSM for the Ukraine represented 0.3% of the male population, with the 'best' estimate with truncation for outliers increasing to 0.7% [435].

Statistical analyses were performed using STATA version 12 (STATA Corp, College Station, TX, USA).

Chapter 6

Determining Components for Estimating HIV Incidence

Within the following chapter I characterise persons newly diagnosed within each country for whom residual samples are available for RITA. For Estonia this includes new diagnoses from all testing centres across the country, for Poland new diagnoses from all 30 VCT sites, and for Kiev, persons testing at the four HIV testing sites Kiev City oblast. I estimate a number of parameters required for estimating incidence, and investigate differences in testing behaviours by sub-populations.

6.1 Characterising persons newly diagnosed with HIV

6.1.1 Kiev City

A total of 6370 adults (3425 men and 2945 women) were tested for HIV at any one of the four HIV testing sites, known as the Kiev AIDS Centres in the Kiev City Oblast between April 2013 and March 2014. This equating to a crude testing rate of 293.2 tests per 100,000 population (aged ≥ 16 years) (345.8 for men and 249.1 for women) (Table 6.1). The median age at test was 30 years (IQR: 26-36), with the majority testing due to symptoms (table 6.2). HIV test results could not be linked for 353 specimens. Of the remaining 6017 tests with a result, 467 (7.8%) were HIV positive, equivalent to a diagnosis rate of 21.5 per 100,000 persons (table 6.1). The completion rate for questionnaires was 99.4% with only 39 persons not providing any further information.

Characteristics		Population [436]	Persons seeking an HIV test	HIV testing rate	New HIV diagnoses	Diagnosis rate
Total		2,172,448	6370	293.2	467	21.5
Age (years)	16-25	484,839	1466	302.4	64	13.2
	26-30	212,340	1795	845.3	120	56.5
	31-35	177,968	1456	818.1	154	86.5
	≥ 36	1,297,301	1653	127.4	129	9.9
Sex	Male	990,412	3425	345.8	303	30.6
	Female	1,182,036	2945	249.1	164	13.9

Source: Department for Population and Regional Statistics of the State Statistics Service of Ukraine [436].

Table 6.1: HIV testing and diagnosis rates (per 100,000 population) for persons seeking an HIV test at any one of the four HIV testing sites between April 2013 - March 2014 in Kiev City, Ukraine.

Probable route of exposure to HIV was available for 5986 of the 6017 (99.5%) individuals with a test result, the majority (4803; 80%) reported heterosexual contact, and 191 (3.2%) identified as MSM (table 6.2). Of the 4803 reporting heterosexual contact, 519 (11%) reported contact with PWID, with a higher proportion for women (417/2587, 16%) compared to men (101/2216, 4.6%). A further 377 persons (7.8%) reported paying for sex or reported that they themselves were paid for sex.

The median age among those newly-diagnosed was higher than those testing (32 years; IQR: 28-36), and HIV prevalence among those testing was highest among persons aged 31-35 years, males, PWID and MSM (table 6.2). The proportion of persons newly-diagnosed among those in the heterosexual contact exposure category was low and similar for men and women. For PWID, the proportion newly-diagnosed was somewhat higher for women compared with men (21.4% vs. 17.1% respectively).

After excluding those who were not resident within Kiev City, both the testing and new diagnosis rate were lower at 265.6 per 100,000 and 17.9, however the proportion of persons newly diagnosed by subpopulations remained unchanged.

	HIV test	Test result known	Newly Diagnosed	
	n = 6370	N = 6017	N = 467	%
Age (years)				
16-25	1466	1381	64	4.6
26-30	1795	1698	120	7.1
31-35	1456	1376	154	11.2
≥ 36	1653	1562	129	8.3
Sex				
Male	3425	3224	303	9.4
Female	2945	2793	164	5.9
Residence				
Kiev (city)	5771	5457	391	7.2
Kiev area (small town)	412	384	53	13.8
Kiev area (village)	111	108	10	9.3
Another area of Ukraine	46	43	7	16.3
Not Reported	30	25	6	
Probable route of exposure				
Persons who inject drugs: Male	835	801	137	17.1
Persons who inject drugs: Female	198	191	41	21.5
Men who have sex with men	201	191	46	24.1
Heterosexual contact: Male	2370	2216	114	5.1
Heterosexual contact: Female	2728	2587	122	4.7
Not reported	38	31	7	
Testing history				
Repeat tester	1413	1365	84	6.2
First-time tester	4787	4499	349	7.8
Not Reported	170	153	34	
Reason for test				
Clinical Indicators	3166	2336	257	11.0
High Risk Behaviour	2427	2977	163	5.5
General Public Screening	735	668	40	6.0
Not Reported	42	36	7	

Table 6.2: Distribtution of all persons testing for HIV and persons newly diagnosed at any one of the four HIV testing sites between April 2013 - March 2014 in Kiev City, Ukraine.

6.1.2 Poland

Preliminary results for 2013, indicate that 32,306 persons presented for an HIV test at one of the 30 VCT centres across Poland, with 17,986 tests among men and 14,320 among women. Table 6.3 shows the corresponding rates with 99.9 tests per 100,000 population (aged ≥ 16 years) (116.3 for men and 84.9 for women).

Characteristics		Population [437]	Persons seeking an HIV test	HIV testing rate	New HIV diagnoses	Diagnosis rate
Total		32,328,338	32,306	99.9	381	1.2
Age (years)	16-19	1,810,315	1413	78.1	4	0.2
	20-29	5,854,195	16,495	281.8	171	2.9
	30-39	6,123,509	10,275	167.8	143	2.3
	≥ 40	18,540,318	4123	22.2	63	0.3
Sex	Male	15,465,990	17,986	116.3	338	2.2
	Female	16,862,348	14,320	84.9	43	0.3

Source: Eurostat [437].

Table 6.3: HIV testing and diagnosis rates (per 100,000 population) for persons seeking an HIV test at a VCT, Poland: 2013.

381 persons were newly diagnosed giving an HIV prevalence of 1.2% with, with the majority (89%; 338) of new diagnoses among being men. 264 (69%) reported sex between men as their risk, followed by 22% reporting heterosexual contact and 9.2% identifying as PWID (table 6.4). Information on overall testing figures were not available by risk group.

	HIV test	Newly Diagnosed	
	n = 32,306	N = 381	%
Age (years)			
16-19	1413	4	0.3
20-29	16,495	171	1.0
30-39	10,275	143	1.4
≥ 40	4123	63	1.5
Sex			
Male	17,986	338	1.9
Female	14,320	43	0.3
Probable route of exposure			
Persons who inject drugs: Male		24	
Persons who inject drugs: Female		11	
Men who have sex with men		264	
Heterosexual contact: Male		50	
Heterosexual contact: Female		32	

Table 6.4: Distribtution of all persons testing for HIV and persons newly diagnosed at a VCT site, Poland: 2013.

6.1.3 Estonia

A total of 320 persons (≥ 18 years) (199 men and 121 women) were newly diagnosed with HIV in Estonia during 2013, equating to a crude diagnosis rate of 29.7 per 100,000 population (40.5 diagnoses for men and 20.7 for women) (table 6.5). The median age at diagnosis was 32 years (IQR: 27-41), with the majority being diagnosed within the capital Tallinn, and testing being due to symptoms or high risk behaviour (table 6.6).

Characteristics		Population [437]	New HIV diagnoses	Diagnosis rate
Total		1,076,483	320	29.7
Age (years)	18-24	119,352	41	34.4
	25-30	115,161	97	84.2
	31-35	90,519	51	56.3
	≥ 36	751,451	131	17.4
Sex	Male	490,847	199	40.5
	Female	585,636	121	20.7

Source: Eurostat [437].

Table 6.5: HIV diagnoses rates (per 100,000 population) for persons newly diagnosed in Estonia during 2013.

Risk factor information was available for 210 (66%), the majority of whom were infected through heterosexual contact (66%). For the 110 perons with no reported risk factor information, 73 (66%) were men.

Among those testing due to screening (n=79), 27 were screened because of incarceration, 19 because of pregnancy, 4 were blood donors and for 29 the screening reason is not known. These persons were excluded from further analysis as testing frequencies and incidence methodologies differ from populations testing due to self initiation, given that population size of those screened are known.

After exclusions, 241 diagnoses were included in analysis, the distribution was similar to that for all diagnoses with a median age of 34 years (IQR: 28-43) (table 6.6).

Characteristics	New HIV Diagnoses	New Diagnoses (excluding persons testing due to screening)
	n= 320	n= 241
Age (years)		
18-24	41	24
25-30	97	64
31-35	51	40
≥ 36	131	113
Median (IQR)	32 (27-41)	34 (28-43)
Sex		
Male	199	156
Female	121	85
Probable route of exposure		
Heterosexual Male	64	48
Heterosexual Female	75	52
Persons Who Inject Drugs	65	40
Men Who have Sex with Men	6	6
Not Reported	110	95
Testing history		
Repeat testers	59	44
Reason for Test		
Clinical Indicators	85	85
High Risk Behaviour	61	61
General Screening	79	
Other	6	6
Not Reported	89	89

IQR, Inter Quartile Range.

Table 6.6: Characteristics of persons newly-diagnosed with HIV during 2013 in Estonia.

6.2 Testing Patterns

The chosen sample framework for this study using existing national surveillance methods within Estonia, the four HIV testing sites in Kiev City and and all VCT sites in Poland will be affected by the test seeking behaviours of individuals. As identified by Remis *et al*, symptoms of primary infection, high risk behaviours and concurrent STI symptoms could lead to increases in testing rates and within an earlier time frame [407, 408].

Such factors could lead to an overestimation of incidence and will be more prominent within certain subgroups. Furthermore, different test seeking behaviours will influence the probability of testing within one year of infection, a component needed for incidence estimates within the sampling framework, with populations testing more frequently having a greater probability of testing within one year of infection than those testing less frequently (Chapter 4).

There is little to no information regarding testing frequencies and behaviours in Kiev City, Estonia or Poland. Using data collected as part of the study for Kiev City and Estonia and through new diagnoses within the VCT sites between 2008 and 2010 for Poland, I examined testing frequencies by subpopulations, to understand factors associated with repeat testing in those countries.

Data regarding previous HIV tests for each country were used to quantify HIV testing rates among the overall population, stratifying by socio-demographic data collected for each individual.

6.2.1 Kiev City

Data described in section 6.1.1 of 6370 adults (16 years and older) attending for an HIV test at any one of the four HIV testing sites in the Kiev City Oblast between April 2013 and March 2014 were analysed. Socio-demographic information, risk factors and testing history were collected using a pre-test questionnaire, discussed in section 5.4.1. Persons reporting a previous test were classified as repeat testers. Persons were excluded if no date of previous test was reported (n=6), the previous test was within one month of the current test (n=45), where the previous test result was not known (n=91). After exclusions, 6200 had information on testing history available, of

whom 1413 (23%) were identified as repeat testers.

Table 6.7 shows the distribution of testing history among persons attending for an HIV test during April 2013 to March 2014. The median age among all persons was 30 years [IQR: 26-36]; 30 years [25-36]; among first-time testers, and 31 years [27-36] among repeat testers.

Characteristics	Attendees	First-time testers (n= 4787)		Repeat testers (n= 1413)	
	n= 6200	n	%	n	%
Age (years)					
<30	2787	2252	81	535	19
≥ 30	3413	2535	74	878	26
Median (IQR)	30 (26-36)	30 (25-36)		31 (27-36)	
Sex					
Male	3326	2543	76	783	24
Female	2874	2244	78	630	22
Residence					
Kiev City	5637	4320	77	1317	23
Outside Kiev City	561	466	83	95	17
Not Reported	2	1	50	1	50
Probable route of infection					
Heterosexual Contact	5012	4017	80	995	20
PWID	985	640	65	345	35
MSM	196	123	63	73	37
Not Reported	7	7	100	0	0
Reason for test					
Clinical Indicators	3092	2429	79	663	21
High Risk Behaviour	2365	1671	71	694	29
General Public Screening	729	682	94	47	6
Not reported	14	5	36	9	64

IQR, Inter Quartile Range.

Table 6.7: Testing history among persons attending the Kiev AIDS centre for an HIV test during the period April 2013 - March 2014.

In univariate logistic regression models, repeat testers were more likely to be older, MSM or PWID, and testing due to high risk behaviour, compared with first time testers (table 6.8). As the interaction between sex and probable route of exposure was found to be significant (p=0.004), I re-categorised these two variables to create a new variable (MSM, Heterosexual contact: Female, Heterosexual contact: Male, PWID: Female, and PWID: Male). After adjusting for all other variables, probable route of exposure/sex remained an independent predictor for repeat testing. Compared to heterosexual women, MSM and PWID (males and females) were more likely to repeat test [Odds Ratio (OR): 1.87; 1.37-2.55 and 1.93; 1.62-2.30 and 1.40; 1.00-1.96, respectively], and heterosexual men were less likely to repeat test [OR=0.78; 0.68-0.91]. Furthermore, repeat testing was significantly more likely with increasing age [OR=1.28 per 10 year increase, 1.19-1.39] and among those engaging in high risk behaviour [OR=1.55; 1.37-1.76] compared with those testing due to clinical indicators. Repeat testing was less likely among those testing as part of screening [OR=0.28; 0.20-0.38] and those resident outside the City [OR=0.66; 0.52-0.84] (table 6.8). Heterosexual women reporting high risk behaviour (high risk partner or sex work) had a higher proportion of repeat testers than heterosexual men overall (25%; 135/539 vs. 19%; 72/374), led predominately by women with partners who inject drugs (28%; 119/420).

	Univariate		Multivariate †	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Age (per 10 year increase)	1.21 (1.12-1.30)	<0.001	1.28 (1.19-1.39)	<0.001
Area of residence				
Kiev City	1	<0.001	1	<0.001
Outside Kiev City	0.67 (0.54-0.85)		0.66 (0.52-0.84)	
Probable route of exposure				
Heterosexual contact: Female	1	<0.001	1	<0.001
Heterosexual contact: Male	0.81 (0.70-0.93)		0.78 (0.68-0.91)	
MSM	2.16 (1.59-2.93)		1.87 (1.37-2.55)	
PWID: Female	1.52 (1.09-2.12)		1.40 (1.00-1.96)	
PWID: Male	2.10 (1.77-2.50)		1.93 (1.62-2.30)	
Reason for test				
Clinical Indicators	1	<0.001	1	<0.001
General Public Screening	0.25 (0.19-0.34)		0.28 (0.20-0.38)	
High Risk Behaviour	1.52 (1.34-1.72)		1.55 (1.37-1.76)	

† Adjusted for all variables in table.

Table 6.8: Factors associated with repeat testing among persons attending the Kiev AIDS centre for an HIV test during the period April 2013 - March 2014.

These findings were further supported when investigating the number of tests individuals reported having in the past 2 years, with persons reporting sexual contact with the opposite sex more likely to report 1 test in the past 2 years, whereas MSM were more likely to report 2 or more (table 6.9).

Characteristics	Repeat testers (n=1413)	Number of tests in the last 2 years			
		1	2	3	≥ 4
Age (years)					
<30	535	289	119	55	29
≥ 30	878	445	205	85	52
Sex					
Male	783	411	184	72	51
Female	630	323	140	68	30
Residence					
Kiev City	1317	676	313	134	73
Outside Kiev City	95	58	11	5	8
Probable route of infection					
Heterosexual Contact	995	531	205	86	54
PWID	345	182	104	34	12
MSM	73	21	15	20	15
Reason for test					
Clinical Indicators	663	373	140	44	30
High Risk Behaviour	694	326	177	94	47
General Public Screening	47	32	4	2	3

Table 6.9: Number of tests reported in the past two years by repeat testers attending the Kiev AIDS centre for an HIV test during the period April 2013 - March 2014.

6.2.2 Poland

I investigated testing frequencies among persons presenting at a VCT site within Poland through analysing data collected by the National AIDS Centre, Warsaw, Poland between January 2008 and December 2010. These data were of all persons newly diagnosed as positive and a random sample of 1000 negative tests from each year.

Data were collected at a person's initial presentation for an HIV test through a pre-test questionnaire and a self-reported questionnaire. Variables collected using the pre-test questionnaire includes socio-demographic information, risk factors and testing history. Variables collected using the self-reported questionnaire includes education, marital status, employment, economic status, and self-health assessment, further details of data collections are found in section 5.4.2.

Data for 3588 adults (16 years and older) attending for an HIV test at one of 30 VCT centres in Poland between 2008 and 2010 were analysed. Persons reporting a previous test were classified as a repeat tester. Persons were excluded if no information on testing history was reported (n=52), if the previous test was positive (n=63) or equivocal (n=27), if the previous test result was not known (n=175), the date of the previous test was not known (n=295) or where the previous test was within the same month as the diagnostic test (n=49). To ensure persons are only counted once, I excluded anyone who reported a previous date between 2008 and 2010 and indicated that the test was performed at the VCT (n=370). After exclusions, 2557 had testing history available, of whom 381 (15%) were identified as repeat testers.

Table 6.10 shows the distribution of testing history among persons attending a VCT clinic between 2008 and 2010. The median age among all persons was 27 years [IQR:23-33], 26 years [23-32] among first-time testers, and 30 years [26-35] among repeat testers.

Characteristics	Attendees †	First-time testers (n= 2176)		Repeat testers (n= 381)	
	n= 2557	n	%	n	%
Age (years)					
<27	1229	1121	91	108	8.8
≥ 27	1313	1042	79	271	21
Not reported	15	13		2	
Median (IQR)	27 (23-33)	26 (23-32)		30 (26-35)	
Sex					
Male	1416	1167	82	249	18
Female	1137	1005	88	132	12
Not reported	4	4		0	
Area of residence					
City	2220	1866	84	354	16
Outside City	224	208	93	16	7.1
Not reported	113	102		11	
Probable route of exposure					
Heretosexual Contact	2055	1801	88	254	12
Persons who inject drugs	125	90	72	35	28
Men who have sex with men	351	261	74	90	26
Not reported	26	24		2	

† Data were of all persons newly diagnosed as positive and a random sample of 1000 negative tests from each year.

Table 6.10: Testing history among persons attending for an HIV testing at a VCT clinic between 2008 and 2010: Poland.

In univariate logistic regression models, repeat testers were more likely to be older, resident in a city, MSM or PWID compared with first-time testers (table 6.11). After adjusting for all factors, probable route of exposure remained an independent predictor for repeat testing. Compared to heterosexual contact, MSM and PWID were more likely to repeat test [Odds Ratio (OR): 2.29; 1.69-3.12) and OR: 2.77 (1.81-4.25) respectively]. Furthermore, repeat testing was significantly more likely with increasing age [OR=1.29 per 10 year increase, 1.15-1.43]. Repeat testing was less likely among those resident outside a city [OR=0.45; 0.27-0.76] (table 6.11).

Characteristics	Univariate		Multivariate †	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Age (per 10 year increase)	1.27 (1.14-1.41)	<0.001	1.29 (1.15-1.43)	<0.001
Sex				
Male	1	<0.001	1	0.19
Female	0.61 (0.48-0.77)		0.84 (0.65-1.09)	
Area of residence				
City	1	<0.001	1	0.001
Outside City	0.42 (0.25-0.71)		0.45 (0.27-0.76)	
Probable route of exposure				
Heterosexual contact	1	<0.001	1	<0.001
Persons who inject drugs	2.86 (1.88-4.36)		2.77 (1.81-4.25)	
Men who have sex with men	2.46 (1.86-3.25)		2.29 (1.69-3.12)	

† Adjusted for all variables in table.

OR, Odds Ratio.

Data were of all persons newly diagnosed as positive and a random sample of 1000 negative tests from each year.

Table 6.11: Predictors for repeat testing among persons attending for an HIV test between 2008 and 2010: Poland.

6.2.3 Estonia

Among the 241 persons newly diagnosed during 2013 within Estonia as described in section 6.1.3, data on a previous test within the past 2 years was requested from confirmatory laboratories using the citizen ID, with a negative test within the past 2 years reported for 35 (15%) newly diagnosed in 2013. The distribution of persons testing negative in the past two years are within table 6.12 with younger adults, women and persons testing because of high risk behaviour more likely to test in the past two years. Adjusting for all variables, there were no independent predictors identified.

Characteristics	New Diagnoses (excluding persons testing due to screening)	A negative test in the past 2 years	
	n = 241	n = 35	%
Age (years)			
18-24	24	8	33
25-30	64	15	23
31-35	40	6	15
≥ 36	113	6	5.3
Median (IQR)	34 (28-43)	28 (25-33)	
Sex			
Male	134	19	14
Female	63	16	25
Probable route of exposure			
Heterosexual Men	48	6	13
Heterosexual Women	52	9	17
Persosn Who Inject Drugs	40	7	18
Men Who have Sex with Men	6	0	
Not Reported	95	13	14
Reason for Test			
Clinical Indicators	85	8	9.4
High Risk Behaviour	61	11	18
Other	6	0	
Not Reported	89	16	18

IQR, Inter Quartile Range.

Table 6.12: Distribution of persons with a negative test reported in the past two years of diagnosis, Estonia 2013.

6.3 Determining Key Parameters for Estimating HIV Incidence

As described in section 4.3, when information on the negative population is not available, the true number of recent infections needs to be estimated using the probability of testing and being classified as recent (P).

For repeat testers, this probability is estimated by the time between a person's last negative and first positive test. For first-time testers, the proportion of persons diagnosed late (with an AIDS diagnosis and/or CD4 cell count <200 cells/mm^3) is used to derive that probability.

The methodology by Karon *et al* [409] estimates the probability of testing and being classified as recent using equation 6.1.

$$P = P_1 \times P_2 \times P_{\mathrm{w}} \tag{6.1}$$

where P_1 is the probability of having an HIV test within the reference period, P_2 is the probability of being tested with a TRI and P_w is the probablity of being classified as recent. For repeat testers P_1 equals 1/T with T representing the time between a person's last negative and first positive result. For first-time testers, P_1 equals $1/\beta$, with β representing the mean time from infection to test. β is estimated using equation 6.2 where q is the proportion diagnosed late and α_A and β_A are the shape and scale parameter, respectively, of the incubation period from infection to an AIDS diagnosis.

$$\beta = \frac{[\beta_A]}{[q^{-1/\alpha_A} - 1]} \tag{6.2}$$

Prejean's [346] method, described in section 4.3.2 modifies this formula by estimating the probability of testing and being classified as recent directly using equation 6.3 for repeat testers and 6.4 for first-time testers. Here Sw(t) is the probability of a person being classified as recent at time t after infection and $S_A(t)$ is the survival function for the AIDS incubation period. These modifications remove the upper limit P_w placed on the probability of testing and considers that a person's inter-test interval could be less than 1 year. I will estimate the probability of testing and being classified as recent using both methodologies to compare the incidence estimates.

$$P = \frac{1}{n}\sum_{i=1}^{T}\frac{1}{T_{\mathrm{i}}}\int_{0}^{T_{\mathrm{i}}} Sw(t)\,dt \tag{6.3}$$

$$P = \int_{0}^{\infty} Sw(t)S_{\mathrm{A}}(t)\frac{1}{\beta}e^{-\mathrm{t}/\beta}\,dt \tag{6.4}$$

6.3.1 Method A: Karon *et al*: Repeat testers

Kiev City

Using data described in section 6.1.1 and section 6.2.1, for persons attending the Kiev AIDS centre for a test between April 2013 and March 2014, testing history was available for 6200, of whom 1413 (23%) were identified as having a repeat test, of whom 482 (34%) had re-tested within a year of their negative test. Among those subsequently diagnosed as HIV positive, the respective figures were as follows: 433, 84 (19%) and 17 (20%). The probability of testing within a year of infection for all repeat testers was 0.70, and for those HIV positive 0.566 (table 6.13). These two probabilities were also estimated for the key characteristics identified as predictors for repeat testing (section 6.2.1) which are needed to control for any heterogeneity in testing frequencies. Populations with a greater probability of testing within a year of infection were: men (0.607), age 16-25 years (0.693), and MSM (0.650) (table 6.13). These probabilities are estimated as the average of 1/T for each repeat tester (see section 4.3.1), with T equal to the interval between the person's last negative test and first positive test. Figure 6.1 shows the distribution of these probabilities for repeat testers newly diagnosed during the time period.

	Testing History	Repeat tester		Re-tested within 1 year		P_1 ‡	95% CI
	n	n	%	n	%		
HIV Positive							
All	433	84	19	17	20	0.566	0.496-0.631
Age (years)							
16-25	61	13	21	5	38	0.693	0.523-0.856
26-30	108	28	26	3	11	0.55	0.457-0.641
31-35	143	30	21	7	23	0.598	0.479-0.717
≥ 36	121	13	11	2	15	0.397	0.235-0.569
Sex							
Male	282	55	20	14	25	0.607	0.526-0.686
Female	151	29	19	3	10	0.487	0.367-0.598
Probable route of exposure							
Persons Who Inject Drugs	162	30	19	5	17	0.535	0.437-0.643
Men Who have Sex with Men	46	13	28	3	23	0.65	0.509-0.790
Heterosexual Contact	225	41	18	9	22	0.561	0.461-0.674

‡ Probability of testing within a year of infection.

CI, Confidence Interval.

Table 6.13: Probability of testing within a year of infection for repeat testers newly diagnosed at the Kiev AIDS centre between April 2013 and March 2014: Ukraine.

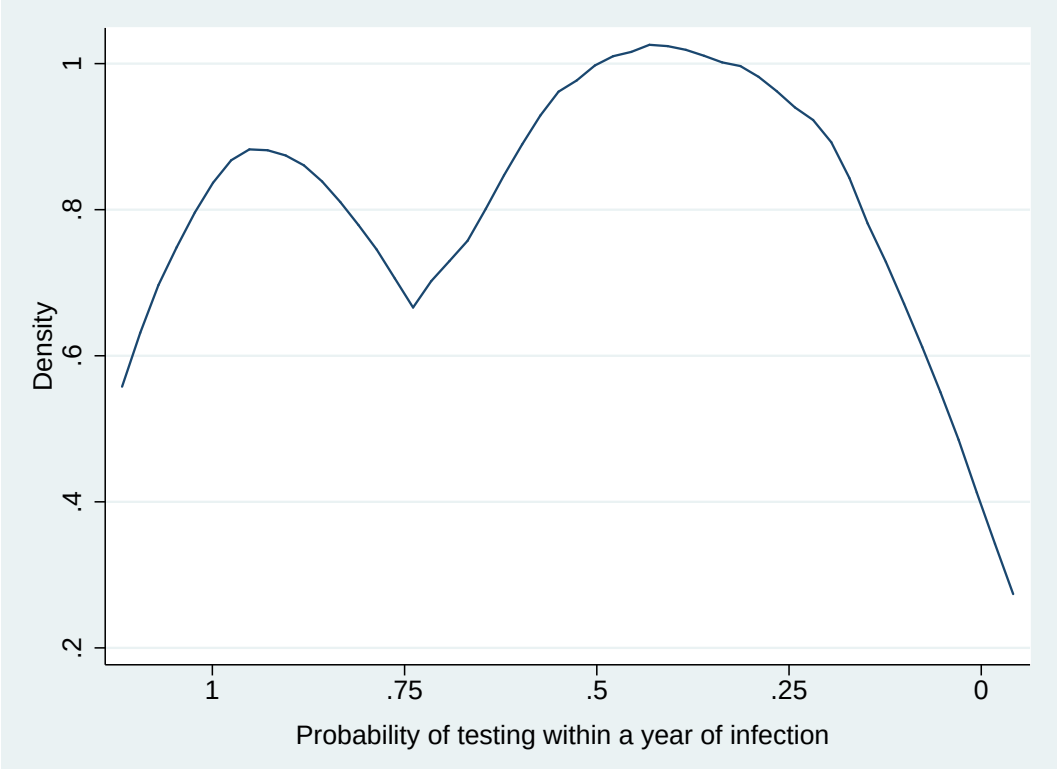


Figure 6.1: Distribution of the probability of testing within a year of infection for repeat testers newly diagnosed at the Kiev AIDS centre April 2013 and March 2014.

To estimate the true number of recent infections within the population (T_r), the number of diagnostic tests identified as recently infected is divided by the probability of being tested and classified as recent (P). This probability is the function of the probability of testing within a year of infection (P_1 equal to 0.566) and the probability of testing recent (P_w). The probability of testing recent or P_w, is equal to $\mu/365$ (see formula 4.7) which is equal to 130 days/365 resulting in a P_w of 0.356. Using formula 6.1, assuming that all new diagnoses have been tested with LAg Avidity EIA, the probability of testing and being classified as recent (P) is equal to 0.201. Therefore, for every one recent infection identified, this probability suggests the true number of recent infections is 4.9, suggesting a further 3.9 recent infections are within the population currently undiagnosed. Figure 6.2 shows both the probability of testing within a year of infection (blue line) and the probability of testing and being classified as recent (red line). This figure illustrates how the probability of testing and being classified as recent is bounded by the probability of being classified as recent, with the blue line reaching a probability of 1 for those who test at yearly intervals with the overall probability (red line) reaching a maximum of 0.356.

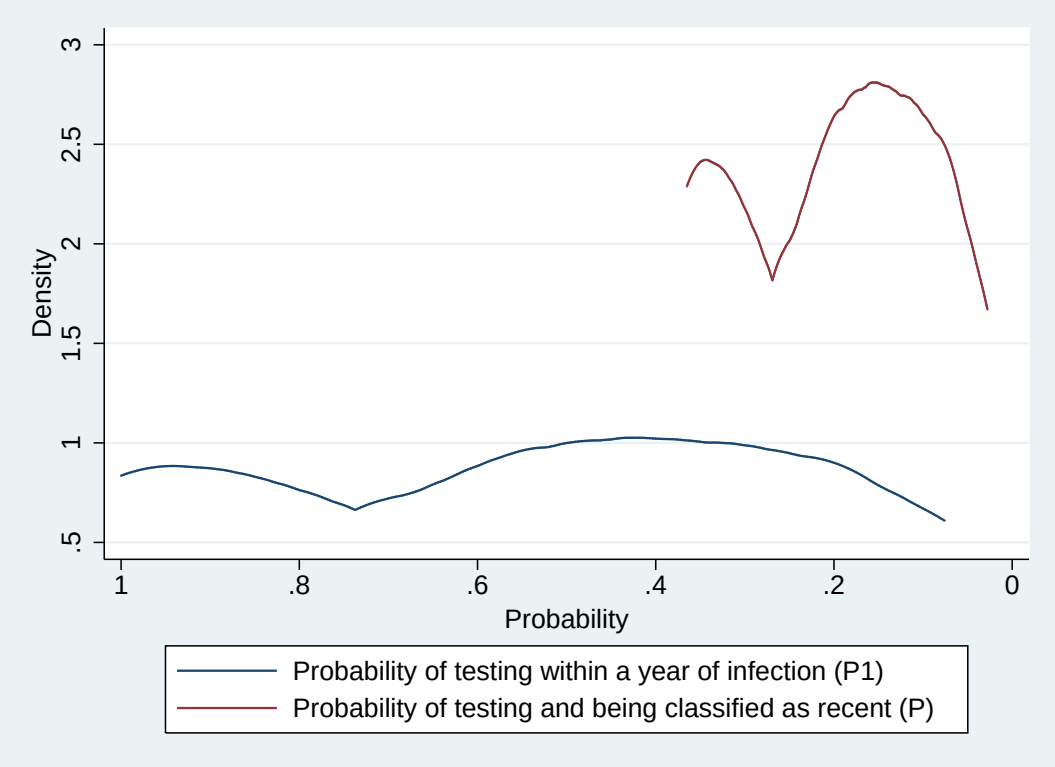


Figure 6.2: Distribution of the probability of testing within a year of infection for repeat testers (blue line) and the probability of testing and being classified as recent (red line) newly diagnosed at the Kiev AIDS centre April 2013 and March 2014.

Poland

Using data from 2008-2010, described in section 6.2.2, testing history and a diagnosis date were reported for 2503 persons, of whom 372 (15%) were repeat testers, of whom 63 (17%) had re-tested within a year of their last negative test date. Among those subsequently diagnosed as HIV positive, the respective figures were as follows: 344, 77 (26%) and 8 (10%). The probability of testing within a year of infection among those diagnosed HIV positive was 0.375 (table 6.14). Probabilities were also estimated for the key characteristics identified as predictors for repeat testing (section 6.2.2) to control for heterogeneity in testing frequencies. Although, older persons ($\geq$ 27 years) were more likely to repeat test , the probability of testing within a year of infection was greater for those age 20-29 years (table 6.14), with a higher probability of testing within a year of infection among MSM compared to those reporting heterosexual contact. These probabilities are estimated as the average of 1/T for each repeat tester (see section 4.3.1), with T equal to the interval between the person's last negative test and first positive test. Figure 6.3 shows the distribution of these probabilities for repeat testers newly diagnosed during the time period.

	Testing History	Repeat tester		Re-tested within 1 year		P_1 ‡	95% CI
	n	n	%	n	%		
HIV Positive							
All	344	77	22	8	10	0.375	0.314-0.444
Age (years)							
16-19	12	0	0	0	0	0	
20-29	147	26	18	5	19	0.508	0.394-0.629
30-39	112	38	34	3	7.9	0.341	0.256-0.437
≥ 40	68	13	19	0	0	0.207	0.145-0.281
Sex							
Male	275	67	24	8	12	0.386	0.322-0.456
Female	69	10	14	0	0	0.298	0.194-0.391
Probable route of exposure							
Persons Who Inject Drugs	52	15	29	3	20	0.382	0.219-0.561
Men Who have Sex with Men	156	42	27	4	10	0.41	0.331-0.501
Heterosexual Contact	128	20	16	1	5.0	0.295	0.214-0.400

‡ Probability of testing within a year of infection.

CI, Confidence Interval.

Table 6.14: Probability of testing within a year of infection for repeat testers newly diagnosed at a VCT clinic between 2008 and 2010: Poland.

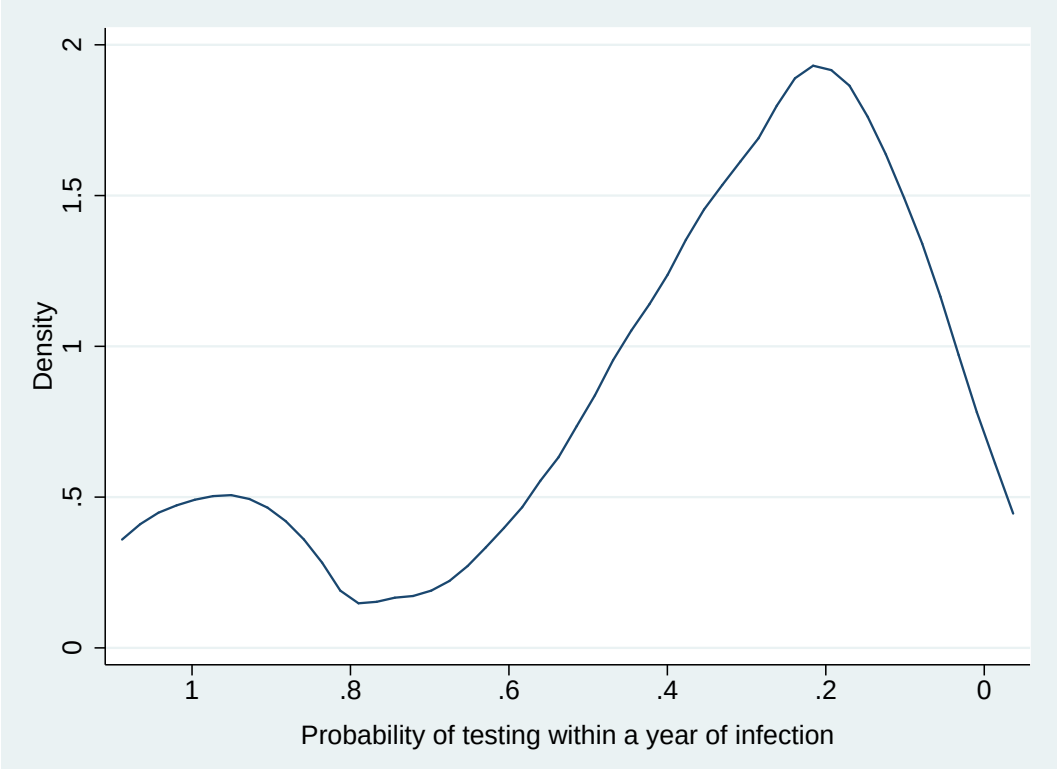


Figure 6.3: Distribution of the probability of testing within a year of infection for repeat testers newly diagnosed at a VCT site across Poland during 2013.

To estmate the true number of recent infections within the population (T_r), the number of diagnostic tests identified as recently infected is divided by the probability of being tested and classified as recent (P). This probability is the function of the probability of testing within a year of infection (P_1 equal to 0.375) and the probability of testing recent (P_w). The probability of testing recent or P_w, is equal to $\mu/365$ (see formula 4.7) which is equal to 130 days/365 resulting in a P_w of 0.356. Using formula 6.1, assuming that all new diagnoses have been tested with LAg Avidity EIA, the probability of testing and being classified as recent (P) is equal to 0.134. Therefore, for every one recent infection identified, this probability suggests the true number of recent infections is 7.5, suggesting a further 6.5 recent infections are within the population currently undiagnosed. Figure 6.4 shows both the probability of testing within a year of infection (blue line) and the probability of testing and being classified as recent (red line). This figure illustrates how the probability of testing and being classified as recent is bounded by the probability of being classified as recent, with the blue line reaching a probability of 1 for those who test at yearly intervals with the overall probability (red line) reaching a maximum of 0.356.

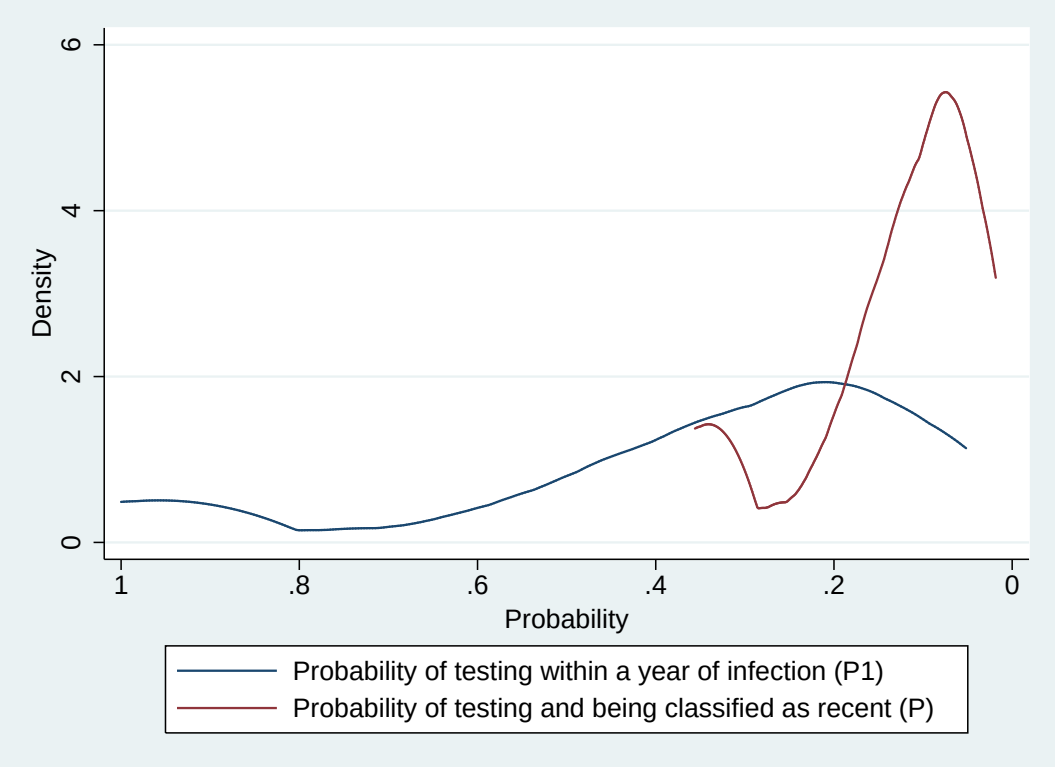


Figure 6.4: Distribution of the probability of testing within a year of infection for repeat testers (blue line) and the probability of testing and being classified as recent (red line) newly diagnosed at a VCT site across Poland during 2013.

Estonia

Using data from new diagnoses within Estonia during 2013, 18% (44) had a previous negative test reported. Due to the nature of how these data were collected, the majority (80%) of these negative tests occurred in the past two years. As a result, the probability of testing within a year is high at 0.685 (table 6.15), stratified by subpopulations there was little difference in these probabilities (table 6.15). All probabilities were much higher than for Poland and Kiev City. Like for Poland and Kiev City these probabilities are estimated as the average of 1/T for each repeat tester (see section 4.3.1), with T equal to the interval between the persons last negative test and first positive test. Figure 6.5 shows the distribution of these probabilities for repeat testers newly diagnosed during the time period.

	New Diagnoses	Previous negative test reported		Re-tested within 2 years		Re-tested within 1 year		P_1 ‡	95% CI
	n	n	%	n	%	n	%		
HIV Positive									
All	241	44	18	35	80	11	25	0.685	0.609-0.762
Age (years)									
18-24	24	10	42	8	80	2	20	0.66	0.500-0.823
25-30	64	17	27	15	88	5	29	0.772	0.664-0.886
31-35	40	8	20	6	75	3	38	0.619	0.396-0.837
≥ 36	113	9	8.0	6	67	1	11	0.607	0.471-0.730
Sex									
Male	134	22	16	19	86	5	23	0.756	0.664-0.845
Female	63	22	35	16	73	6	27	0.614	0.502-0.729
Probable route of exposure									
Persons Who Inject Drugs	40	7	18	7	100	1	14	0.826	0.736-0.908
Men Who have Sex with Men	6	0	0	0	0	0	0	0	0
Heterosexual Contact	100	21	21	15	71	6	29	0.633	0.503-0.748

‡ Probability of testing within a year of infection.

CI, Confidence Interval.

Table 6.15: Probability of testing within a year of infection for repeat testers newly diagnosed in Estonia during 2013.

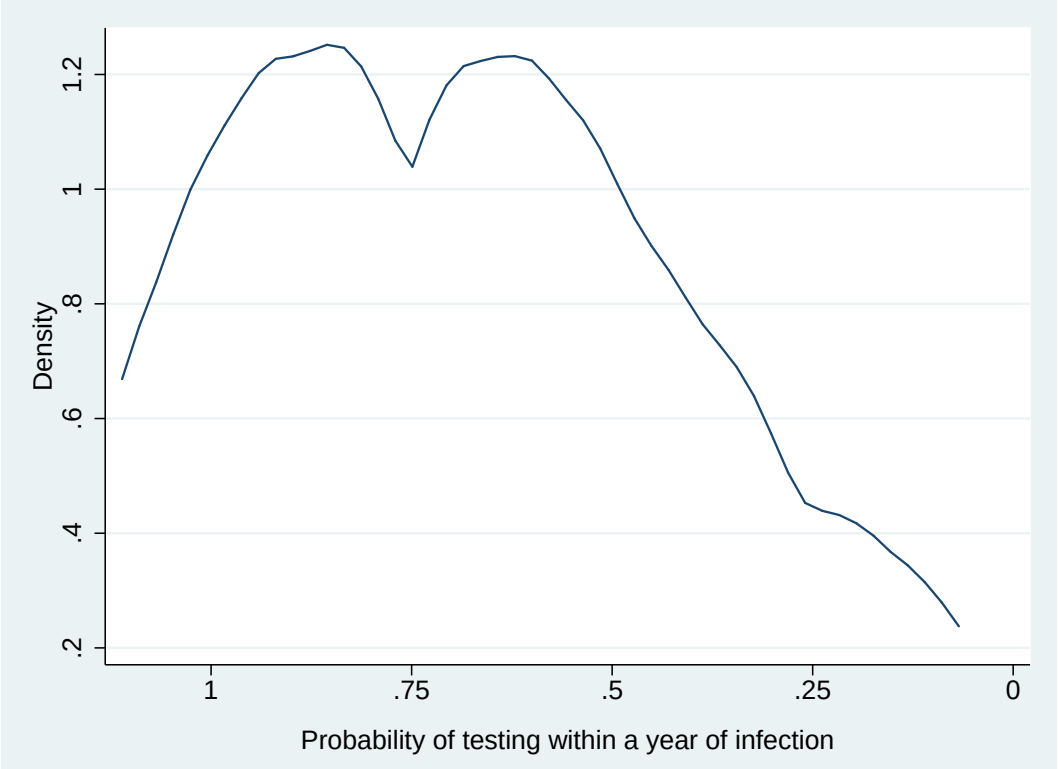


Figure 6.5: Distribution of the probability of testing within a year of infection for repeat testers newly diagnosed across Estonia during 2013.

To estimate the true number of recent infections within the population (T_r), the number of diagnostic tests identified as recently infected is divided by the probability of being tested and classified as recent (P). This probability is the function of the probability of testing within a year of infection (P_1 equal to 0.685) and the probability of testing recent (P_w). The probability of testing recent or P_w, is equal to $\mu/365$ (see formula 4.7) which is equal to 130 days/365 resulting in a P_w of 0.356. Using formula 6.1, assuming that all new diagnoses have been tested with LAg Avidity EIA, the probability of testing and being classified as recent (P) is equal to 0.244. Therefore, for every one recent infection identified, this probability suggests the true number of recent infections is 4.1, suggesting a further 3.1 recent infections are within the population currently undiagnosed. Figure 6.6 shows both the probability of testing within a year of infection (blue line) and the probability of testing and being classified as recent (red line). This figure illustrates how the probability of testing and being classified as recent is bounded by the probability of being classified as recent, with the blue line reaching a probability of 1 for those who test at yearly intervals with the overall probability (red line) reaching a maximum of 0.356.

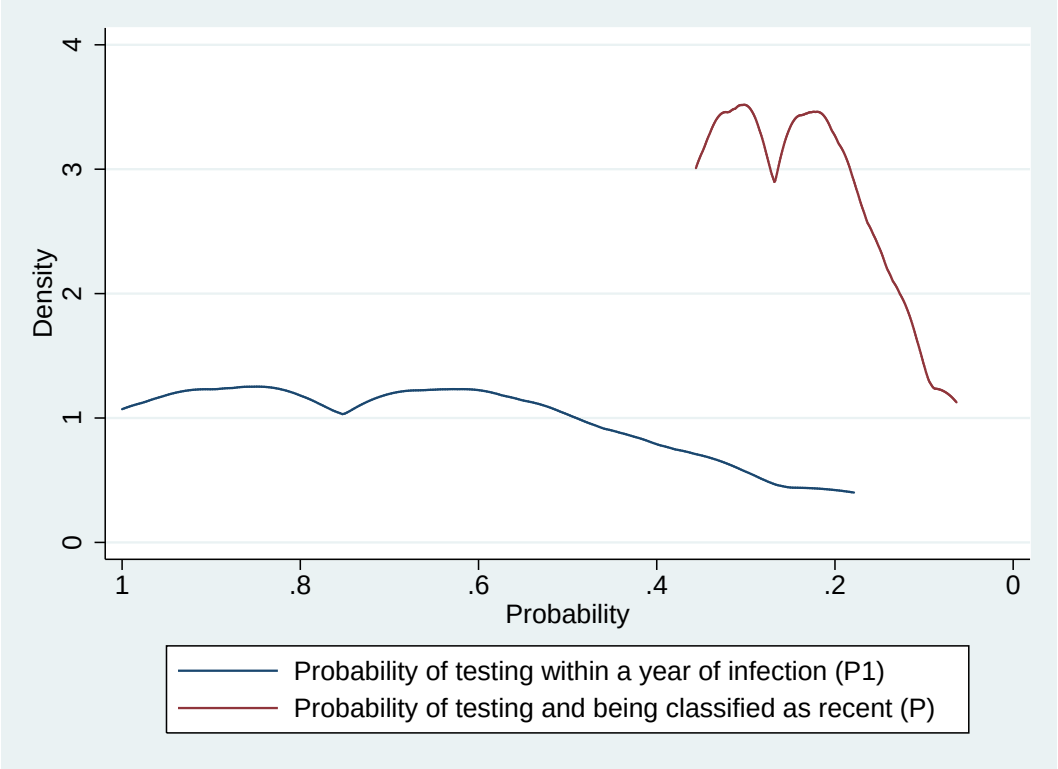


Figure 6.6: Distribution of the probability of testing within a year of infection for repeat testers (blue line) and the probability of testing and being classified as recent (red line) newly diagnosed across Estonia during 2013.

6.3.2 Method A: Karon *et al*: First-time testers

Kiev City

During the first quarter of 2013, 311 persons who were newly-diagnosed with HIV in the Kiev City oblast region attended the Kiev AIDS Centre for care. WHO clinical stage was available for all persons accessing HIV care, 300 (96%) of whom also had a CD4 cell count. Of those with a CD4 cell count reported, 130 (43%) were diagnosed with a CD4 cell count <200 cells/mm^{3}and/or classified as clinical stage IV. Formula 6.5 from section 4.3.1 was used to estimate the probability of testing within 1 year of infection ($1/\beta$).

$$
\begin{aligned}
where\ \beta &= \frac{[\beta_A]}{[q^{-1/\alpha_A}-1]} \\
\Rightarrow \beta &= \frac{[4]}{[0.43^{-1/2}-1]} \\
\therefore \beta &= 7.6 \\
\therefore 1/\beta &= 0.132
\end{aligned}
\tag{6.5}
$$

The 43% diagnosed late (q) resulted in an estimated mean time between infection and HIV testing (β) of 7.6 years, and the probability of a person within this group presenting for an HIV test within 1 year of infection ($1/\beta$), equal to 0.132.

Estonia

Between 2010 and 2012, 656 persons were newly-diagnosed and took up a referral for care in Estonia (248, 240 and 168 for 2010, 2011 and 2012, respectively). CD4 cell count was reported for 158, 176 and 128 persons respectively of whom 36% (57), 27% (46) and 37% (48) had a CD4 cell count <200 cells/mm^3 within three months of HIV diagnosis. Five persons were diagnosed with AIDS within 3 months of HIV diagnosis, of whom one person diagnosed in 2011 had a CD4 >200

cells/mm^3.

The average proportion of persons newly-diagnosed and accessing HIV-related care with a CD4 cell count <200 cells/mm^3 and/or an AIDS diagnosis within 3 months of diagnosis between 2010 and 2012 was 33% (table 6.16).

$$where\ \beta = \frac{[\beta_A]}{[q^{-1/\alpha_A} - 1]}$$

$$\Rightarrow \beta = \frac{[4]}{[0.33^{-1/2} - 1]} \tag{6.6}$$

$$\therefore \beta = 5.36$$

$$\therefore 1/\beta = 0.187$$

Therefore, the estimated probability of testing within 1 year of infection, for those without testing history ($1/\ \beta$), is equal to 0.187.

	New HIV Diagnoses			CD4 Reported						CD4 <200 cells/mm^3						Probability of testing	
	2010	2011	2012	2010		2011		2012		2010		2011		2012		within a year of infection	
	n	n	n	n	%	n	%	n	%	n	%	n	%	n	%	P_1	95% CI
All	248	240	168	158	64	173	72	130	77	57	36	46	27	48	37	0.185	0.156-0.238
Sex																	
Male	156	149	116	94	60	97	65	89	77	40	43	31	32	39	44	0.145	0.103-0.213
Female	92	91	52	64	70	76	84	41	79	17	27	15	20	9	22	0.270	0.179-0.435

CI, Confidence Interval.

Table 6.16: Proportion of persons diagnosed with a CD4 cell count <200 cells/mm^3, by year of diagnosis and sex; Estonia.

6.3.3 Method B: Prejean *et al*

Alternatively, the probability of testing and being classified as recent can be estimated directly for both repeat and first-time testers using the methodology described in section 4.3.2. For repeat testers, the probability of testing and being classified as recent (P) was estimated as 0.305, 0.192 and 0.368 for Kiev City, Poland and Estonia respectively, and for first-time testers 0.044 for Kiev City and 0.075 for Estonia. Data for Poland are not currently available for first-time testers. The probability of testing and being classified as recent (P) for all repeat testers newly diagnosed and by subpopulations are shown in table 6.17 for Kiev City, table 6.18 for Estonia and 6.19 for Poland, with the distribution shown in figures 6.7, 6.8 and 6.9.

	Probability of testing and being classified as recent			
	Repeat Testers	**95% CI**	**First-time Testers**	**95% CI**
HIV Positive				
All	0.305	0.257-0.358	0.056	0.046-0.066
Sex				
Male	0.332	0.268-0.405	†	†
Female	0.252	0.178-0.333	†	†
Age Group				
16-25	0.387	0.257-0.525	†	†
26-30	0.28	0.210-3.65	†	†
31-35	0.32	0.235-0.411	†	†
≥ 36	0.238	0.111-0.418	†	†
Probable Route of Exposure				
Persons who inject drugs	0.271	0.210-0.347	†	†
Men who have sex with men	0.328	0.238-0.439	†	†
Heterosexual Contact	0.322	0.235-0.416	†	†

CI, Confidence Interval.

†Data is not yet available for these populations

Table 6.17: Direct estimation of probabilities of testing and being classified as recent by Subpopulations for Kiev City.

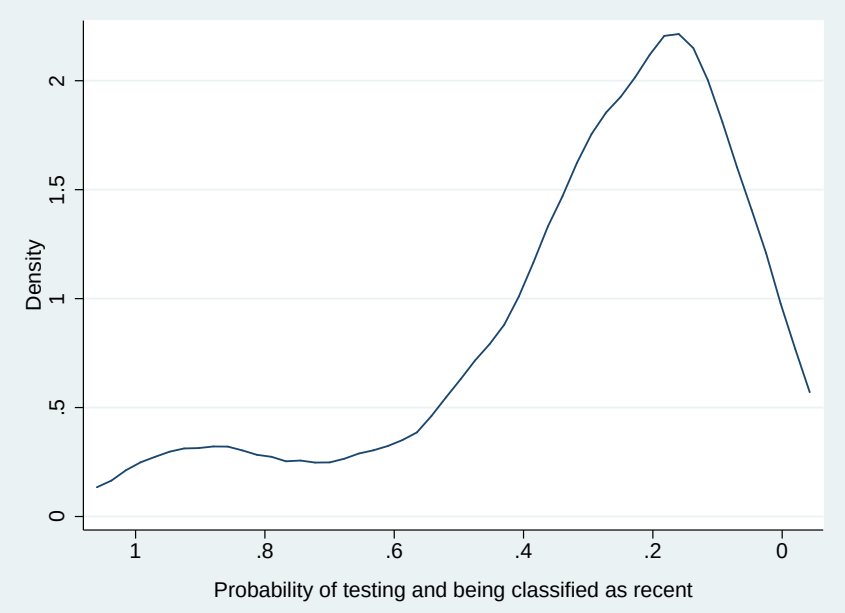


Figure 6.7: Distribution of the probability of testing and being classified as recent among repeat testers newly diagnosed at the Kiev AIDS centre April 2013 and March 2014.

	Probability of testing and being classified as recent			
	Repeat Testers §	**(95% CI)**	**First-time Testers**	**(95% CI)**
HIV Positive				
All	0.368	0.305-0.433	0.077	0.066-0.099
Sex				
Male	0.402	0.321-0.492	0.061	0.044-0.089
Female	0.333	0.245-0.434	0.111	0.075-0.172
Age Group				
18-24	0.314	0.221-0.413	†	†
25-30	0.434	0.333-0.543	†	†
31-35	0.361	0.192-0.554	†	†
≥ 36	0.308	0.212-0.442	†	†
Probable Route of Exposure				
Persons who inject drugs	0.409	0.334-0.522	†	†
Men who have sex with men	‡		†	†
Heterosexual Contact	0.359	0.254-0.469	†	†

CI, Confidence Interval. †Data is not yet available for these populations

‡There were no repeat testers among those reporting MSM as the probable route of exposure.

Table 6.18: Direct estimation of probabilities of testing and being classified as recent by Subpopulations for Estonia.

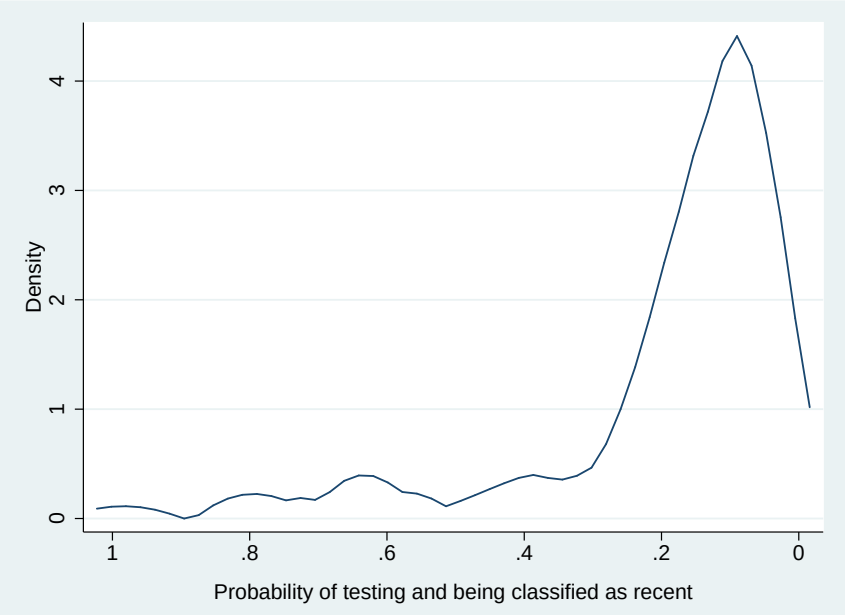


Figure 6.8: Distribution of the probability of testing and being classified as recent among repeat testers newly diagnosed at 30 VCT sites across Poland during 2013.

	Probability of testing and being classified as recent			
	Repeat Testers	**(95% CI)**	**First-time Testers**	**(95% CI)**
HIV Positive				
All	0.192	0.153-0.237	†	†
Sex				
Male	0.201	0.151-0.253	†	†
Female	0.132	0.089-0.179	†	†
Age Group				
16-19	0	0	†	†
20-29	0.266	0.186-0.353	†	†
30-39	0.176	0.124-0.248	†	†
≥ 40	0.091	0.065-0.120	†	†
Probable Route of Exposure				
Persons who inject drugs	0.227	0.107-0.367	†	†
Men who have sex with men	0.204	0.151-0.264	†	†
Heterosexual Contact	0.14	0.093-0.209	†	†

CI, Confidence Interval. †Data is not yet available for these populations

Table 6.19: Direct estimation of probabilities of testing and being classified as recent by Subpopulations for Poland.

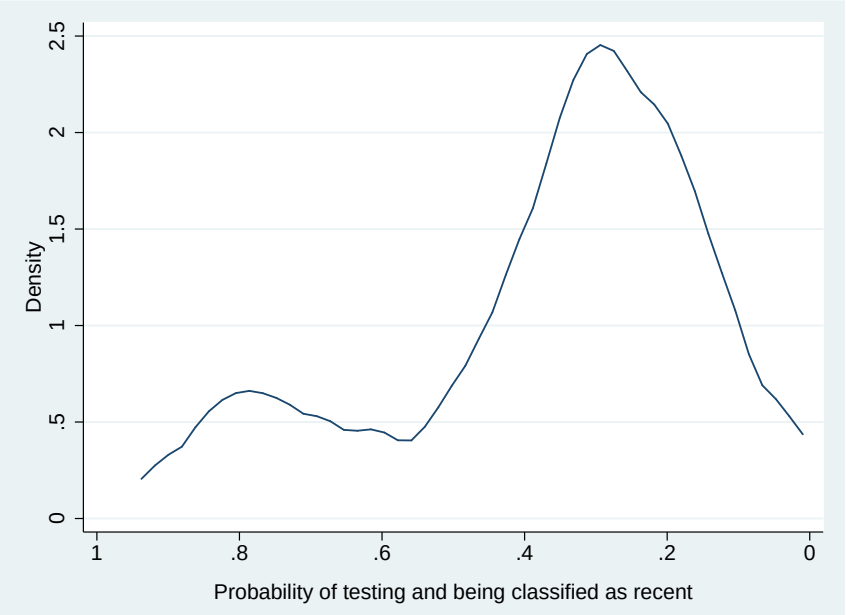


Figure 6.9: Distribution of the probability of testing and being classified as recent among repeat testers newly diagnosed in Estonia during 2013.

Using the modified method by Prejean *et al* [346] the probabilities of testing and being classified as recent are estimated directly. However, with the initial methodology this probability is the function of two probabilities; the probability of testing within a year of infection, estimated in section 4.3.2, and the probability of being classified as recent, estimated by dividing the MDRI for the assay over the reference period. For this study, the MDRI for the LAg Avidity EIA is 130 days and the reference period is 365 days, resulting in a probability of 0.365. Table 6.20 gives the probability of testing and being classified as recent using the two methodologies, with those estimated using the Prejean method higher than those using the Karon method [346, 409].

	Kiev City				Poland				Estonia			
	Karon *et al*[§]		Prejean *et al*		Karon *et al*[§]		Prejean *et al*		Karon *et al*[§]		Prejean *et al*	
	RT	**FTT**	**RT**	**FTT**	**RT**	**FTT**	**RT**	**FTT**	**RT**	**FTT**	**RT**	**FTT**
HIV Positive												
All	0.201	0.047	0.305	0.056	0.134	†	0.192	†	0.244	0.066	0.368	0.077
Sex												
Male	0.216	†	0.332	†	0.137	†	0.201	†	0.269	0.052	0.402	0.061
Female	0.173	†	0.252	†	0.106	†	0.132	†	0.219	0.096	0.333	0.111
Age Group												
16-25 [‡‡]	0.247	†	0.387	†	0	†	0	†	0.235	†	0.314	†
26-30 [‡‡]	0.196	†	0.28	†	0.181	†	0.266	†	0.275	†	0.434	†
31-35 [‡]	0.213	†	0.32	†	0.121	†	0.176	†	0.220	†	0.361	†
≥ 36 [‡]	0.141	†	0.238	†	0.074	†	0.091	†	0.216	†	0.308	†
Probable Route of Exposure												
Persons who inject drugs	0.190	†	0.271	†	0.136	†	0.227	†	0.294	†	0.409	†
Men who have sex with men	0.231	†	0.328	†	0.146	†	0.204	†	0	†	–	†
Heterosexual Contact	0.200	†	0.322	†	0.105	†	0.14	†	0.225	†	0.359	†

RT: Repeat testers. FTT: First-time testers.

[‡‡] For Estonia this age group was for 18-24 and for Poland 16-19, and 25-30, 20-29, respectively.

[‡] For Poland age groups were 30-39, and ≥ 40.

[§] Probability of testing and being classified as recent = Probability of testing within a year of infection * Probability of being classified as recent.

[†] Data is currently not available for these populations.

Table 6.20: Estimated probability of testing and being classified as recent for new HIV diagnoses and subpopulations. Kiev City, Poland and Estonia.

6.4 Discussion

An HIV prevalence of 7.8% was estimated for persons presenting for an HIV test in Kiev City, equivalent to 21.5 diagnoses per 100,000 population, and an overall testing rate of 293.2 per 100,000 population. For the first time, detailed information on HIV risk behaviour was available, indicating that MSM and PWIDs were disproportionately affected by HIV. Furthermore, given the proportions among newly-diagnosed female heterosexuals reporting sexual contact with PWID, there is further evidence of bridging in risk between PWID and their sexual partners. The diagnosis rate for Estonia was 24.9 per 100,000 population and, although heterosexual contact was the leading route of infection, a third of persons newly diagnosed did not have a risk factor recorded, the majority of whom were men. Finally, preliminary data for Poland indicate that the diagnosis rate for persons attending a VCT site in Poland in 2013 is 1.2 per 100,000 population, with the majority reporting their risk as MSM.

Although, the diagnosis rate for Kiev City of 21.5 is lower than that for Ukraine as a whole (37.1 per 100,000), it is however, substantially higher than rates for Western and Central Europe (6.6 and 2.1, respectively) [15]. Even though ECDC suggest testing rates are higher for countries in Eastern Europe, the testing rate for Kiev City was one of the lowest in Europe with only Greece reporting a lower rate with 190 per 100,000 population [15].

For the first time detailed information on HIV risk behaviour was available, indicating that MSM and PWID were disproportionately affected by HIV. Although the majority of persons presenting for an HIV test reported heterosexual contact, one in five MSM and PWID testing for HIV were diagnosed positive. Using MSM population estimates discussed in section 5.8 [434, 435], the diagnosis rate for MSM ranged from 490.6/100,000 to 1548.3/100,000. This is substantially higher than the diagnosis rates of 76.6 to 241.7/100,000 suggested by the 152 MSM diagnosed in Ukraine overall [15], though the prevalence of MSM may differ between Kiev and the rest of Ukraine.

Interestingly, there was little difference in the diagnosis rates between men and women reporting heterosexual contact. Due to high rates of stigma and discrimination within Ukraine I had anticipated non-disclosure of homosexuality to be high, with men more likely to report they were a heterosexual male, leading to a higher rate of HIV diagnosis in heterosexual men.

However, this was not reflected in the data, suggesting that non-disclosed homosexuality among men is unlikely to be substantial. However, findings were in agreement with previous findings suggesting a bridging between high risk individuals and their partners with an estimated one in six heterosexual women reporting contact with a PWID.

Testing patterns among persons presenting for an HIV test in Kiev City are consistent with current literature [271, 407, 408, 412–414], with those at high risk of HIV more likely to test frequently, with probable route of exposure (MSM and PWID) and reason for test (high risk behaviour) all being independently associated with repeat testing. Age was also associated with repeat testing, which may, in part, be due to younger persons having had less time to test than those who are older. Around one in three MSM and PWID reported a previous test compared to one in five among heterosexuals, indicating that persons at risk have some awareness about testing. This was also evident among heterosexual women with partners who inject drugs. This increase in testing is likely to result in a person presenting for a test closer to their date of HIV infection, and has a direct impact on the probability of testing within a year of infection, highlighting the importance of controlling for these difference among subpopulations when estimating incidence at a sub-population level.

This probability of testing within a year of infection as described in section 4.3, uses the testing frequencies of persons who have tested more than once or the population for those presenting for a test for the first-time, to establish whether the number of recent infections collated through new diagnosis is a true reflection of the number of recent infections within the population. Among repeat testers, the more frequently a person tests the more likely they are to be diagnosed when recently infected. Among those self-identifying as a repeat tester by reporting a previous negative test in Kiev City, one in five tested positive within a year of their last negative test. The respective probability of testing within a year of infection was 0.566, suggesting that a high proportion of persons recently infected will have presented for an HIV test close to their time of infection, and that the number classified as recent is close to the true number recent. The probability of 0.566 suggests that for every 1 recent infection a further 0.8 recent infections have not yet been diagnosed.

Alternatively, among first-time testers, the proportion of the population who test because

they have had their infection for many years is used to estimate the likelihood this population will test close to infection. A population with a low proportion of persons diagnosed late (CD4 cell count <200 cells/mm^3) suggests a shorter average time between infection and diagnosis than a population with a high proportion of late diagnosis. The proportion diagnosed late within Kiev City was estimated to be 43%, suggesting an 8 year gap between infection and diagnosis and a probability of testing within the first year of 0.132. This proportion will be much higher if persons reporting a previous negative test were removed from the population, with a longer gap between infection and diagnosis.

Data on CD4 and clinical stage at diagnosis are not widely available within Ukraine, and the data used were collected from patients' notes for the first quarter of 2013. Although data are reflective of the study period, it is not possible to state whether they are typical of recent years. In Europe on average, where CD4 completion was >75% for each country, the average CD4 cell count <200 cells/mm^3 at diagnosis, for countries within the EU/EEA was 26%, whereas among non-EU/EEA countries this proportion was much higher at 43% [15]. This high proportion among non-EU/EEA countries was comparable to that in Kiev City. Furthermore, the probability of testing within a year of infection 0.132 was also comparable to those for the USA [409] and Italy [348] with 0.240 and 0.136 respectively. This lower probability indicates that for every person with no testing history who is classified as recent there are 6.6 more recent infections.

As previously discussed, because of differences in testing patterns among subpopulations, I estimated the probability of testing within a year of infection for repeat testers separately by age group, sex and probable route of exposure. Where the probability of testing within a year was higher than the overall population, such as for males and MSM with 0.607 and 0.650, respectively, the incidence estimate would be overestimated if the overall 0.566 probability was used. Likewise, as this probability lower for females and persons diagnosed aged ≥ 36 years (0.487 and 0.397, respectively) their incidence estimate would be an underestimate. Like the repeat testers, the proportion diagnosed late and, therefore, the probability of testing within a year, is likely to differ by subpopulations. Higher rates of testing among MSM and PWID suggests that MSM are less likely to be diagnosed late compared to persons exposed through heterosexual contact [15], leading to a higher number of undiagnosed heterosexuals compared to MSM. However, these data are not currently available within Kiev City.

The probability of testing and being classified as recent (P) can be estimated directly using the methodology in section 4.3.2, rather than estimating the probability of testing within a year and the probability of being classified as recent separately. The probability of testing in the first year was consistently higher among repeat testers, rather than first-time testers as expected, with a higher probability among men (0.332), MSM (0.328) and PWID (0.322). Estimating this probability directly allows repest testers to have an inter-test interval of less than a year and allow for a recency period of greater than a year, preventing an underestimation of P. This is shown when comparing Karon's P of 0.201 for repeat testers and 0.047 for first-time testers with Prejean's of 0.305 and 0.056, respectively.

The 24.9 per 100,000 population diagnosis rate for Estonia with 320 persons newly diagnosed in 2013, was consistent with the 2012 diagnoses rates published by ECDC (23.5 per 100 000) [15]. Data for Estonia, are collected initially through the Estonian Health board and then through the HIV database once persons attend for care. As all new diagnoses within Estonia were collected this meant there was data for those self-initiated testing and for those being screened. These testing populations are very different, and use very different methods for estimating incidence, which is why persons testing through screening were excluded from any further analysis.

Among those persons who presented for a test, excluding those testing due to screening, two thirds reported their probable risk of HIV as heterosexual contact, however a third of persons newly diagnosed did not have a risk factor recorded, of which the majority were men. With the distribution of sex for Estonia mirroring that of Kiev with 3 in 5 new diagnoses being among men, but with a higher proportion of diagnoses among MSM and PWID, we can consider that those without a risk factor could later report these risk groups.

Data on whether a person has tested previously were collected directly from 5 out of the 6 major laboratories within Estonia which represent an estimated 80-90% of the total tests performed [Pilleriin Soodla, personal communication, August, 2014]. Although these negative tests are more reliable than self-reported information, the majority of data only reflect the past two years. As a result, the probability of testing within a year among these persons was much higher with 80% re-testing within 2 years and 25% within 1 year. There was little difference by

subgroup, with a higher probability among men (0.756), persons aged 25-30 (0.772) and PWID (0.826). However, there were no independent predictors for repeat testing.

To estimate the proportion of persons diagnosed late in Estonia, I used data available from the previous 3 years (2010-2012). Where a CD4 cell count was available (70%), on average a third of persons were diagnosed with a CD4 less than 200 cells/mm^3, resulting in a probability of testing and being classified as recent of 0.077. As indicated previously, the average proportion of persons diagnosed late in EU/EEA regions was 26%, lower that the 30% for Estonia. Although, among the repeat testers men had a higher probability of testing within a year of infection, this population also had a higher proportion of late diagnosis compared with women resulting in a lower probability of testing and being cllassified as recent of 0.063 compared with 0.111. This difference could be due to antenatal screening among women resulting in women being diagnosed earlier in their infection.

Finally, due to data processing procedures within Poland (a commercial company is tasked with data entry) a completed dataset is not available until the end of the following year. However, preliminary data indicate that the diagnosis rate for persons attending a VCT site in Poland in 2013 is 1.2 per 100,000 population, lower than the 2.8 reported for the whole of Poland in 2012. However, this difference is likely to reflect the difference in populations, with this study only including persons testing at a VCT. From the preliminary data, the distribution of persons at risk differed significantly from that of Kiev City and Estonia, with nine of ten persons newly diagnosed at a VCT being male, with the majority of diagnoses being among MSM.

Data from persons testing at a VCT site from previous years (2008-2010) were used to understand testing patterns and estimate the probability of testing within a year of infection among repeat testers. Testing patterns were also consistent with published literature [271, 407, 408, 412–414], with both MSM and PWID more likely to repeat test, and with higher odds of testing with increasing age. The probability of testing within a year of infection for repeat testers was much lower than in Kiev City, Italy and the USA [346, 348], with only 1 in 10 persons re-testing within a year of their last negative test.

Estimating the probability by subpopulation was consistent with testing patterns with MSM

having a higher probability of testing within a year compared with heterosexuals (0.410 vs. 0.295). However, although increasing age was associated with repeat testing, the highest probability was found among those aged 20-29 years. This indicates that repeat testing and age is a factor of how long they have had to repeat test and does not necessarily reflect how regularly they test. Of note, re-testing was less likely among persons living outside cities, which could suggest that testing facilities are less accessible outside cities, or a lower understanding of the importance of testing among persons in those settings.

Chapter 7

Estimating Incidence

Within the following chapter I present the distribution of persons classified as recent within each country. I estimate HIV incidence for all persons and for sub-populations where information is available. I explore differences in incidence, based on the availability of data components identified for estimating incidence, and testing behaviour.

Through nucleotide sequencing I investigated the distribution of HIV subtype and evidence of transmitted drug resistance among persons newly diagnosed with HIV, in particular those recently infected.

7.1 Characterising Persons Recently Infected

7.1.1 Odessa

I analysed data from an initial study conducted within Odessa during 2009, where residual samples from all newly-diagnosed HIV positive individuals (≥ 16 years) across Odessa city and province between May and December 2009 were tested for evidence of recent infection using the laboratory assay known as the BED-CEIA. See section 5.4.1 for methods.

1,315 persons aged ≥ 16 years were tested with the BED assay, of whom 2 were excluded as they were subsequently discovered to be HIV negative. Of the remaining 1,313, 321 (24%) were classified as recent. Median age at HIV diagnosis was 32 years [inter quartile range (IQR): 26-39], with men accounting for 54% of diagnoses. The majority of persons tested for HIV were part of general public screening (42%) or due to clinical indications (36%) (table 7.1).

Table 7.1 shows the distribution of newly-diagnosed individuals and the number identified as recently-infected. Younger adults appeared to be more likely to be identified as recently-infected ($p<0.001$), as did women ($p=0.013$). Those resident in the city of Odessa were less likely to be identified as recently-infected ($p=0.019$). The interaction between sex and residence was found to be significant ($p=0.015$). I, therefore, re-categorised and created a new term of sex and residence.

	New HIV diagnoses	Recently infected		p-value
		n	%	
Age at HIV diagnosis (years)				
<25	209	87	*42*	
25-30	343	91	*27*	<0.001
31-40	488	87	*18*	
>40	273	56	*21*	
Sex				
Male	709	154	*22*	0.013
Female	604	167	*28*	
Residence				
Odessa: City	692	149	*22*	
Odessa: Rural	550	156	*28*	0.019
Non-resident in Odessa	54	15	*28*	
Not Reported	17	1	*5.9*	
Reason for test				
Clinical Indicators	404	93	*25*	
High Risk Behaviour	341	88	*24*	0.78
General Public Screening	557	138	*25*	
Other	11	2	*18*	
Total	1,313	321	*24*	

Table 7.1: Characteristics of persons newly-diagnosed with HIV and the proportion identified as recent by the BED assay in Odessa, Ukraine: May-December 2009.

Findings were largely confirmed in multivariable logistic regression models (table 7.2). Age remained an independent predictor for recent infection with increasing age being associated with a lower probability (OR=0.70 per 10-year increase, 95% CI= 0.60-0.82). Compared to men residing in Odessa city, women in rural Odessa (1.85, 1.26-2.71) and non-resident men (2.83, 1.15-6.97) were more likely to be recently-infected. Reason for test was not independently associated with recent infection.

Among those with testing history, none of the persons who reported a negative test within a year of their diagnosis were classified as recent. Of those who were classified as recent four out of the five reported their negative test within two years of their diagnosis.

		Univariable odds ratio for testing recent (95% confidence interval)		p value	Multivariable odds ratio for testing recent† (95% confidence interval)		p value
Age years (per 10 year increase)		0.67	(0.58,0.78)	<0.001	0.7	(0.60,0.82)	<0.001
Residence							
Odessa: City	Male		1	<0.001		1	0.008
	Female	1.36	(0.95,1.96)		1.28	(0.88,1.85)	
Odessa: Rural	Male	1.3	(0.70,1.88)		1.18	(0.81,1.72)	
	Female	2.2	(1.53,3.18)		1.85	(1.26,2.71)	0.008
Non-resident in	Male	3.52	(1.46,8.46)		2.83	(1.15,6.97)	
Odessa	Female	0.78	(0.29,2.10)		0.65	(0.24,1.78)	
Reason for test							
Clinical Indicators		0.91	(0.67,1.23)		1.16	(0.84,1.60)	
High Risk Behaviour		1.06	(0.78,1.45)		1.19	(0.86,1.64)	
General Public Screening			1	0.77		1	0.62
Other		0.67	(0.14,3.12)		0.66	(0.13,3.25)	

† Adjusted for all other factors in table.

Table 7.2: Factors associated with testing recent according to the BED assay in Odessa, Ukraine: May-December 2009.

By May 2011 of the 1,313 newly-diagnosed individuals, 819 (62%) had presented at an HIV/AIDS centre, and information on clinical status was available on 809 of whom 322 (40%) were diagnosed with AIDS and 181 (22%) were known to have died (table 7.3). Among the 321 who were identified by the RITA assay as recently-infected, 209 (65%) presented at an HIV/AIDS centre, 207 of whom had information on clinical status. Of these, 32 (15%) had been diagnosed with AIDS and 23 (11%) were known to have died. Among those deemed to have been recently-infected, 21 (66%) of those diagnosed with AIDS, and 15 (65%) of those known to have died, had had an HIV test due to clinical indications.

When I excluded the 404 individuals who were tested due to clinical indications, 228 (25%) of the remaining 909 were classified as recent. Findings from the logistic regression analyses remained qualitatively unchanged with older age being associated with lower probability of testing as recently-infected (OR=0.69 per 10 year increase, 0.56-0.84).

	Did not re-attend	Re-attended: Clinical status				Vital Status
		Asymptomatic	Symptomatic (non-AIDS)	AIDS	Not Recorded	Died
	n=494(%)	n=396(%)	n=26(%)	n=322(%)	n=75(%)	n=181(%)
Clinical indication						
Diagnosed with a clinical indicator	185 (37)	32 (8)	4 (15)	126 (39)	54 (72)	103 (57)
Died	3 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
High risk behaviour						
Heterosexual contact	17 (3)	52 (13)	6 (23)	24 (8)	0 (0)	5 (3)
Injecting Drug User	54 (11)	30 (8)	4 (15)	45 (14)	7 (9.3)	24 (13)
Sex between men	0 (0)	1 (0.3)	0 (0)	1(0.3)	0 (0)	1 (0.6)
Occupational risk	1 (0.2)	5 (1)	0 (0)	2 (0.6)	0 (0)	1 (0.6)
Multiple partners	7 (1)	8 (2)	0 (0)	4 (1)	0 (0)	0 (0)
Diagnosed with an Sexually transmitted Disease	34 (7)	26 (7)	3 (12)	7 (2)	3 (4)	5 (3)
General public screening						
Blood Donors	21 (4)	23 (6)	1 (4)	4 (1)	1 (1)	1 (0.6)
Prisoner	49 (10)	17 (4)	3 (12)	17 (5)	7 (9)	9 (5)
Antenatal Screening	44 (9)	126 (32)	0 (0)	3 (0.9)	1 (1)	1 (0.6)
Military Recruitment	3 (0.6)	4 (1)	1 (4)	0 (0)	0 (0)	0 (0)
Regulations/policy[†]	70 (14)	70 (18)	4 (15)	86 (27)	2 (3)	31 (17)
Other						
Anonymous testing	4 (0.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Foreign citizen	1 (0.2)	0 (0)	0 (0)	2 (0.6)	0 (0)	0 (0)
Not recorded	1 (0.2)	2 (0.5)	0 (0)	1 (0.3)	0 (0)	0 (0)

[†] For employment, visa applications and medical care workers who deal with blood.

Table 7.3: Characteristics of persons newly-diagnosed with HIV and the proportion identified as recent by the BED assay in Odessa, Ukraine: May-December 2009.

7.1.2 Kiev City

The LAg avidity EIA was conducted on residual samples from persons newly diagnosed with HIV at any one of the four HIV testing sites in Kiev City between April 2013 and March 2014. Figure 7.1 shows the data flow used to classify whether a person has been recently infected. For 21 individuals a sample was not available for testing, of the remaining 446, 39 (8.7%) were initially classified as recent with an avidity index <1.5 ODn. Additional information on viral load was used to minimise risk of misclassification of longstanding infections as recent. Of those with an avidity score of <1.5, 10 had a viral load <1000 copies/ml and were not considered as recent, resulting in 29 (6.5%) recent HIV infections, 6.6% among repeat testers and 6.5% among first-time testers.

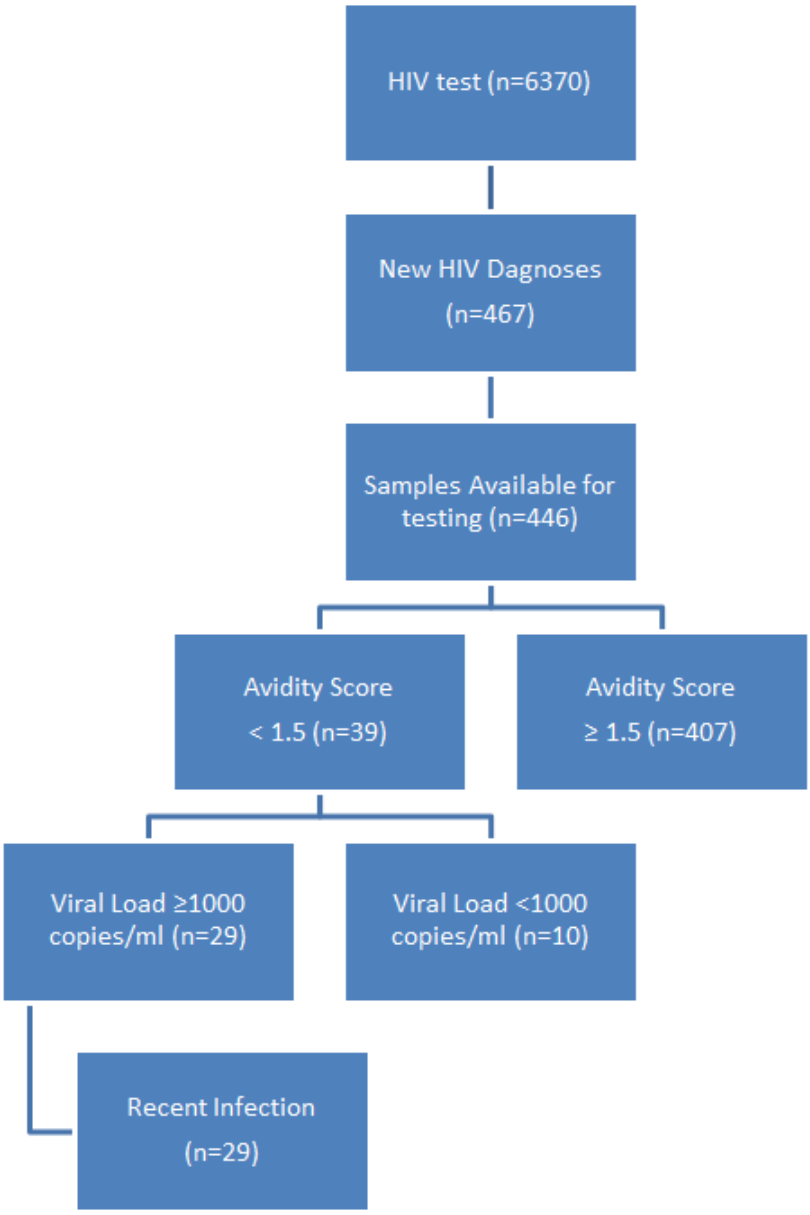


Figure 7.1: Flow of data from presenting for an HIV test four testing sites to being classified as a recent HIV infection, in Kiev City: April 2013 and March 2014.

Median age among those recently infected was 30 years [IQR:24-34], similar to that among all new diagnoses, with men accounting for two thirds of recent infections. Table 7.4, shows the distribution of persons classified as recently infected in Kiev City. Recent infections were more likely to be identified among younger adults and men who have sex with men, with those resident outside Kiev City less likely to be identified as recent.

	Newly Diagnosed		Available Sample	Recent classification	
	N = 467	%	N = 446	N = 29	%
Age (years)					
16-25	64	4.6	61	8	13
26-30	120	7.1	115	10	8.7
31-35	154	11.2	144	8	5.6
≥ 36	129	8.3	126	3	2.4
Sex					
Male	303	9.4	288	23	8.0
Female	164	5.9	158	6	3.8
Residence					
Kiev (city)	391	7.2	373	28	7.5
Kiev area (small town)	53	13.8	51	1	2.0
Kiev area (village)	10	9.3	10	0	0
Another area of Ukraine	7	16.3	6	0	0
Not Reported	6		6		
Probable route of exposure					
Persons who inject drugs: Male	137	17.1	130	3	2.3
Persons who inject drugs: Female	41	21.5	40	1	2.5
Men who have sex with men	46	24.1	43	14	33
Heterosexual contact: Male	114	5.1	109	5	4.6
Heterosexual contact: Female	122	4.7	117	5	4.3
Not reported	7		7	1	
Testing history					
Repeat tester	84	6.2	76	5	6.6
First-time tester	349	7.8	338	22	6.5
Not Reported	34		32	2	
Reason for test					
Clinical Indicators	257	11	248	20	8.1
High Risk Behaviour	163	5.5	154	8	5.2
General Public Screening	40	6	37	1	2.7
Not Reported	7		7		

Table 7.4: Characteristics of persons newly-diagnosed with HIV and the proportion identified as recently infected using the LAg Avidity EIA, Kiev City, Ukraine: April 2013 - March 2014.

Adjusting for all variables in a multivariable logistic regression model indicated that the only independent predictor for being a recently infected was risk, with MSM more likely to present risk compared with persons reporting heterosexual contact (table 7.5).

Where exposure category was reported, MSM accounted for 50% (14/28) of recent infections with heterosexual men accounting for 18% (n=5) and heterosexual women 18% (n=5). The majority of MSM classified as recent fell into the age group 26-30 years (57%; 8/14) and tested due to high risk behaviour (71%; 10/14). Persons who inject drugs represented 14% (n=4) of recent infections, of whom the majority (75%) were male.

Of the 17 persons reporting a negative test within a year of their HIV diagnosis none were found to be recently infected suggesting that the probability of testing within a year of infection for repeat testers is an overestimation which will underestimate the true number of recent infection for that population.

The proportion of persons who maintain an optical density below the assay threshold for greater than a year of infection after correcting for viral load was estimated using residual samples from 109 persons known to have had HIV infection for greater than a year. Initial classification using the LAg Avidity assay, indicated that 6.4% (n=7) were misclassified as recent, dropping to a false recent rate of 3.7% (n=4) after correcting for a viral load <1000 copies/ml.

	Univariable odds ratio for testing recent (95% confidence interval)		p value	Multivariable odds ratio for testing recent † (95% confidence interval)		p value
Age years (per 10 year increase)	0.48	(0.25,0.94)	0.023	0.65	(0.32,1.33)	0.217
Sex						
Male		1	0.133		1	0.928
Female	0.51	(0.20,1.29)		0.95	(0.28,3.17)	
Residence						
Kiev City		1	0.039		1	0.141
Outside Kiev City	0.19	(0.03,1.45)		0.27	(0.03,2.14)	
Probable route of exposure						
Heterosexual contact		1	<0.001		1	<0.001
Persons who inject drugs	0.61	(0.18,2.01)		0.59	(0.16,2.10)	
Men who have sex with men	11.05	(4.39,27.82)		8.86	(2.65,29.6)	
Testing History						
First-time tester		1	0.960		1	0.256
Repeat tester	1.03	(0.38,2.80)		0.76	(0.25,2.30)	
Reason for test						
Clinical Indicators		1	0.236		1	0.621
High Risk Behaviour	0.57	(0.23,1.38)		0.47	(0.18,1.25)	
General Public Screening	0.31	(0.04,2.39)		0.46	(0.06,3.80)	

† Adjusting for all factors in table.

Table 7.5: Factors associated with testing recent according to the LAg assay in Kiev City, Ukraine: April 2013 - March 2014.

7.1.3 Estonia

The LAg Avidity EIA was conducted on residual samples from persons newly diagnosed within Estonia during 2013; persons tested due to screening were excluded from analysis. Figure 7.2 shows the data flow used to classify whether a person has been recently infected, for 23 individuals a sample was not available for testing, and of the remaining 218, 95 (44%) were initially classified as recent with an avidity index <1.5 ODn. Of those with an initial recent infection a viral load was available for 35, of which 2 had a viral load <1000 copies/ml.

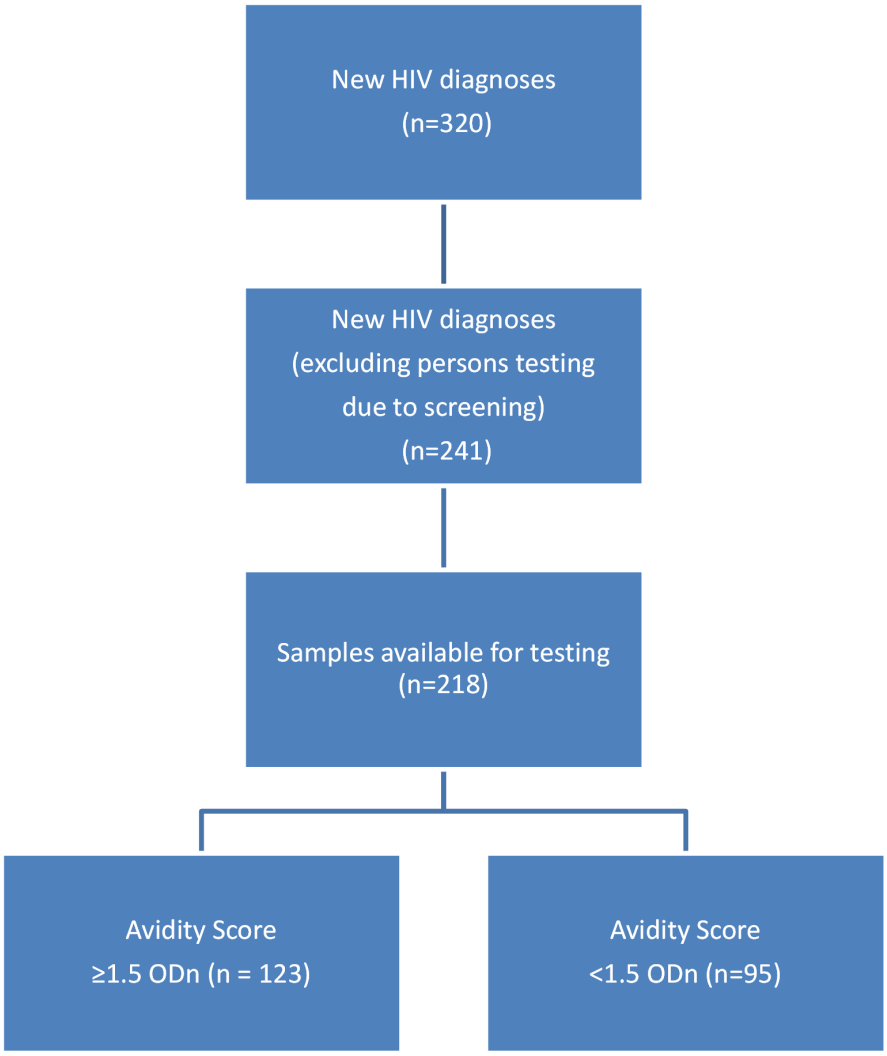


Figure 7.2: Flow of data from presenting for an HIV test to being classified as a recent HIV infection, Estonia: 2013.

Median age among those recently infected was 32 years [IQR:27-42], with men accounting for two thirds of recent infections. Table 7.6, shows the distribution of persons initially classified as recently infected in Estonia. Recent infections were more likely to be among men and those reporting heterosexual contact. Adjusting for all variables in a multivariable logistic regression model there were no independent predictors for being a recent infection.

Five persons reported a seroconversion illness, who were all classified as recent (three had a negative HIV test within the previous year). The 11 persons with a negative test within one year of HIV diagnoses were all correctly classified as recently infected.

The proportion of persons who maintain an optical density below the assay threshold for greater than a year of infection after correcting for viral load was estimated using residual samples from 85 persons known to have had HIV infection for greater than a year. Initial classification using the LAg Avidity assay, indicated that 16% (n=14) were misclassified as recent, dropping to 8.2% (n=7) after correcting for a viral load <1000 copies/ml. Two further persons were identified as elite controllers with a viral load <1000 or 0 and normal CD4 cell count over the years [Pilleriin Soodla, personal communication, August, 2014], resulting in a final false recent rate of 5.9% (5/85).

Characteristics	New Diagnoses †	Available Sample	Initial LAg Avidity score <1.5 ‡	
	n=241	n=218	n=95	%
Age (years)				
16-24	24	23	17	74
25-30	64	58	24	41
31-35	40	35	20	57
≥ 36	113	102	34	33
Median (IQR)	34 (28-43)		32 (27-42)	
Sex				
Male	156	138	58	42
Female	85	80	37	46
Probable route of exposure				
Heterosexual Men	48	39	18	46
Heterosexual Women	52	51	24	47
Persons Who Inject Drugs	40	38	17	45
Men who have Sex with Men	6	5	2	40
Not Reported	95	85	34	40
Testing history				
Repeat testers	44	40	30	75
Reason for Test				
Clinical Indicators	85	75	33	44
High Risk Behaviour	61	57	28	49
Other	6	5	1	20
Not Reported	89	81	33	41

† Excluding persons testing due to screening.

‡ Not yet corrected for viral load.

IQR, Inter Quartile Range.

Table 7.6: Characteristics of persons newly-diagnosed with HIV and the proportion initially identified as recently infected using the LAg Avidity EIA: Estonia 2014.

7.1.4 Poland

The LAg Aividity EIA was conducted on residual samples from all persons newly diagnosed with HIV at a VCT centre across Poland during 2013. Figure 7.3 shows the data flow used to classify whether a person has been recently infected. For 135 individuals a sample was not available for testing, and of the remaining 246, 73 (30%) were initially classified as recent with an avidity index <1.5 ODn. Additional information on viral load was not yet available to minimise the risk of misclassification of long standing infections as recent.

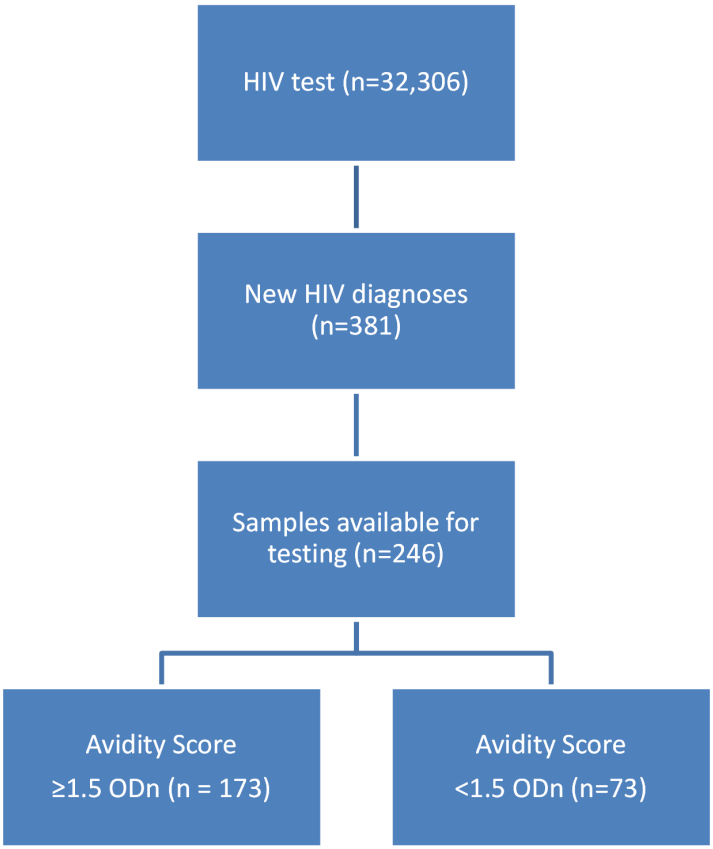


Figure 7.3: Flow of data from presenting for an HIV test at a VCT centre to being classified as a recent HIV infection, in Poland: 2013.

7.2 Incidence Estimates

7.2.1 Kiev City

HIV incidence was initially estimated using the original methodology by Karon *et al* where the true number of recent infections are extrapolated from the observed number using the probability of testing and being classified as recent (P). This methodology estimates P as a factor of the probability of testing within a year of infection and the probability of being classified as recent. I estimated incidence before and after correcting for viral load and used a 20-fold imputation method to address where LAg result and testing history were missing from the data.

Before correcting for viral load, 799 new infections were estimated to have occurred in Kiev City between April 2013 and March 2014, based on the 36 persons classified as recent with testing history. These new infections resulted in an incidence estimate of 36.8 per 100,000 (table 7.7). After correcting for viral load, the number of estimated new infections decreases to 576, equivalent to 26.5 per 100,000 population (table 7.8).

After correcting missing testing history and LAg Avidity result, the estimate using the Karon *et al* methodology was 562 new infections, equivalent to 25.9 new infections per 100,000 population.

The incidence estimate is calculated by dividing the number of new diagnoses classified as LAg recent by P (the probability of testing and being classified as recent), where P is the product of P_1 by P_2 by P_w.

	Original Data		Imputed Data [§]	
	Repeat testers	**First-time testers**	**Repeat testers**	**First-time testers**
New diagnoses	76	338	92	375
LAg Recent (n)	5	31	6	36
P_1	0.566	0.132	0.566	0.132
P_2	0.905	0.968	1	1
P_w [†]	0.356	0.356	0.356	0.356
P	0.182	0.046	0.201	0.047
True recent infections	27	681	30	766
(95% CI)	(25-31)	(576-833)	(27-33)	(648-936)
Incidence estimate	**799**		**796**	
(95% CI)	**(678-974)**		**(674-970)**	
Incidence rate[‡]	**36.8**		**36.6**	
(95% CI)	**(31.2-44.8)**		**(31.0-44.7)**	

[†] 130/365 = 0.356

[‡] Per 100,000 population.

[§] mean values from 20 imputations, described in section 5.8.

CI, Confidence Interval. Confidence intervals were calculated using bootstrapping methologies described in section 4.3.4.

Table 7.7: Incidence estimates for Kiev City, April 2013 - March 2014, before correcting for misclassification of long-standing infections as recent. Karon *et al* Methodology.

	Original Data		Imputed Data [§]	
	Repeat testers	**First-time testers**	**Repeat testers**	**First-time testers**
New diagnoses	76	338	92	375
LAg Recent (n)	5	22	6	25
P_1	0.566	0.132	0.566	0.132
P_2	0.905	0.968	1	1
P_w[†]	0.356	0.356	0.356	0.356
P	0.182	0.046	0.201	0.047
True recent infections	27	483	30	532
(95% CI)	(25-32)	(409-591)	(28-34)	(450-650)
Incidence estimate	**576**		**562**	
(95% CI)	**(489-702)**		**(477-684)**	
Incidence rate[‡]	**26.5**		**25.9**	
(95% CI)	**(22.5-32.0)**		**(22.0-31.5)**	

[†] 130/365 = 0.356

[‡] Per 100,000 population.

[§] mean values from 20 imputations, described in section 5.8.

CI, Confidence Interval. Confidence intervals were calculated using bootstrapping methologies described in section 4.3.4.

Table 7.8: Incidence estimates for Kiev City, April 2013 - March 2014, after correcting for misclassification of long-standing infections as recent. Karon *et al* Methodology.

I also applied the modified methodology by Prejean *et al* where the probability of testing and being classified as recent (P) is estimated directly to ensure that the number of new infections is not overestimated as a result of an underestimated P (see section 4.3.2). For repeat testers P is equal to 0.305 compared to 0.201 using the Karon *et al* Method and 0.056 vs. 0.047 for first-time testers.

I calculated the same results as previously, by dividing the number of new diagnoses classified as LAg recent by P, this resulted in 643 new infections before correcting for viral load, equating to 29.6 new infections per 100,000 (table 7.9), and 462 (21.3/100,000) after correcting for viral load (table 7.10).

After the imputation procedure the final estimates for Kiev City were 466 new infections equating to 21.5 new infections per 100,000 population.

	Original Data		Imputed Data [§]	
	Repeat testers	**First-time testers**	**Repeat testers**	**First-time testers**
New diagnoses	76	338	92	375
LAg Recent (n)	5	31	6	36
P	0.305	0.056	0.305	0.056
True recent infections	16	554	20	643
(95% CI)	(14-19)	(470-674)	(17-23)	(545-783)
Incidence estimate	**643**		**663**	
(95% CI)	**(546-782)**		**(562-806)**	
Incidence rate [‡]	**29.6**		**30.5**	
(95% CI)	**(25.1-36.0)**		**(25.9-37.1)**	

[‡] Per 100,000 population.

[§] mean values from 20 imputations, described in section 5.8.

CI, Confidence Interval. Confidence intervals were calculated using bootstrapping methologies described in section 4.3.4.

Table 7.9: Incidence estimates for Kiev City, April 2013 - March 2014, before correcting for misclassification of long-standing infections as recent. Prejean *et al* Methodology.

	Original Data		Imputed Data [§]	
	Repeat testers	**First-time testers**	**Repeat testers**	**First-time testers**
New diagnoses	76	338	92	375
LAg Recent (n)	5	22	6	25
P	0.305	0.056	0.305	0.056
True recent infections	16	393	20	446
(95% CI)	(14-19)	(333-478)	(17-23)	(379-543)
Incidence estimate	**462**		**466**	
(95% CI)	**(392-561)**		**(396-567)**	
Incidence rate [‡]	**21.3**		**21.5**	
(95% CI)	**(18.0-25.8)**		**(18.2-26.1)**	

[‡] Per 100,000 population.

[§] mean values from 20 imputations, described in section 5.8.

CI, Confidence Interval. Confidence intervals were calculated using bootstrapping methologies described in section 4.3.4.

Table 7.10: Incidence estimates for Kiev City, April 2013 - March 2014, after correcting for misclassification of long-standing infections as recent. Prejean *et al* Methodology.

Using the modified method by Prejean *et al*, I estimated incidence for subpopulations. Although a higher proportion of recent infections were identified among persons aged 16-25 years, incidence estimates were highest among those aged 26-30 years and 31-35 years (both with 72.5 per 100,000). Men had the highest incidence estimates with 34.3 for men compared with 10.2 for women.

Using population estimates for MSM and PWID (discussed in section 5.8), incidence was estimated to be between 2289.6 and 6868.7 per 100,000 MSM and for PWID 350.4. These incidence estimates were significantly higher than that for the overall population. Groups with incidence estimates lower than the overall population were women with 10.2 per 100,000 women and persons ≥ 36 years at 4.3 (table 7.11).

To address for differences in testing patterns as discussed in section 4.3.5, I used population specific probabilities in table 6.20. Although information on testing history was available for all subpopulations allowing for population specific probabilities, the distribution of late diagnosis to determine population specific probabilities for first-time testers is not. Therefore, I used the overall probability of testing and being classified as recent for first-time testers for subpopulations to estimate the true number of recent infections. Where the proportion of late diagnosis is greater than the overall population, this probability will underestimate the number of new infections among first-time testers, and overestimate if the proportion is less.

Characteristics	Population	Incidence Estimate		Incidence Rate †	
	n	n	(95% CI)	n	(95% CI)
Total	2,172,448	466	396-567	21.5	18.2-26.1
Age (years)					
16-25	484,839	121	102-148	25.0	21.0-30.5
26-30	212,340	154	130-188	72.5	61.2-88.5
31-35	177,968	129	109-159	72.5	61.2-89.3
≥ 36	1,297,301	59	50-71	4.5	3.9-5.5
Sex					
Male	990,412	340	288-414	34.3	29.1-41.8
Female	1,182,036	120	102-147	10.2	8.6-12.4
Probable route of exposure					
Persons who injects drugs	23,400	82	70-100	350.4	299.1-427.4
Men who have sex with men	2970			6868.7	5791.2-8451.2
	6930	204	172-251	2943.7	2482.0-3621.9
	8910			2289.6	1930.4-2817.1
Heterosexual contact	‡	159	135-195	‡	‡

† per 100,000 population.

‡ Population level data not available.

CI, Confidence Interval. Confidence intervals were calculated using bootstrapping methologies described in section 4.3.4.

Table 7.11: Incidence estimates for Kiev City, April 2013 - March 2014, by sub-populations. Prejean *et al* Methodology.

7.2.2 Estonia

The same methodologies were used to estimate incidence for Estonia. Viral loads were not available for correcting misclassified results, therefore incidence estimates were based on the 95 diagnoses initially classified as recent using the LAg Avidity EIA.

Incidence estimates for Estonia in 2013, before correcting for misclassification of long-standing infections as recent, was 126.1 per 100,000 population using the original method with 1357 new infections in 2013 and 95.0 using the direct method of estimating P with 1023 new infections (table 7.12). Incidence estimates for men were significantly higher than among women with 170.9 vs. 44.1 respectively.

Methods for estimating incidence are the same as Kiev City, where the number classified as recent is divided by the probability of testing and being classified as recent (P).

	Repeat testers	First-time testers
New diagnoses	40	178
LAg Recent (n)	30	65
Original Method		
P_1	0.685	0.185
P_2	0.909	0.904
P_w †	0.356	0.356
P	0.222	0.06
True recent infections	135	1092
(95% CI)	(122-152)	(849-1295)
Incidence estimate	**1357**	
(95% CI)	**(1073-1600)**	
Incidence rate ‡	**126.1**	
(95% CI)	**(99.7-148.6)**	
Modified Method		
P	0.368	0.077
True recent infections	82	844
(95% CI)	(69-98)	(657-985)
Incidence estimate	**1023**	
(95% CI)	**(802-1197)**	
Incidence rate ‡	**95**	
(95% CI)	**(74.5-111.2)**	

†130/365 = 0.356

‡Per 100,000 population.

CI, Confidence Interval. Confidence intervals were calculated using bootstrapping methologies described in section 4.3.4.

Table 7.12: Incidence estimates before correcting for misclassification of long-standing infections as recent, Estonia, 2013.

7.3 Molecular Epidemiology

7.3.1 Circulating HIV Sub-type

Kiev City

As of September 2014, genotyping, based on the amplification of the HIV-1 pol region, was complete for 118 specimens of the 467 new diagnoses identified in Kiev City between April 2013 and March 2014. The majority (n=116) had a RITA result, of which 15 were among those classified as recently infected, and 101 long-standing.

Using the REGA subtyping tool [438], the majority (92%; 109) of those newly diagnosed persons were infected with subtype A, with the remaining nine classified as subtype B. Table 7.13 shows the distribution of subtype among those newly diagnosed. Eight of the nine persons infected with subtype B were male, of whom 3 reported their risk as MSM, 3 as heterosexual contact and 1 as PWID.

Characteristics	Subtype A n=109	Subtype B n=9
Sex		
Male	73	8
Female	36	1
Probable route of exposure		
Persons Who Inject Drugs	41	1
Men who have Sex with Men	17	3
Heterosexual Men	20	3
Heterosexual Women	27	1
Not Reported	4	1
Recent HIV infection		
Yes	13	2
No	94	7
Not Known	2	0

Table 7.13: Subtype distribution for newly diagnosed persons for whom an HIV nucleotide sequence was available: Kiev City April 2013 - March 2014.

When evaluating the LAg avidity EIA, CEPHIA reported a higher FRR than the recommended 2% when using specimens with Subtype A1, and a longer MDRI of 211 days compared to the 130 days as per the test insert [Gary Murphy, personal communication, March, 2013]. This longer MDRI would increase the probability of being classified as recent and therefore reduce incidence estimates

7.3.2 Transmitted Drug Resistance

Resistance of HIV to antiretroviral drugs is one of the most common causes for therapeutic failure in people infected with HIV. Identifying whether a person has a resistant virus to a particular antiretroviral is an important part of managing HIV, ensuring that the individual is managed in the most effective way possible. Resistance to a particular antiretroviral drug is the result of the random mutations in their genetic structures during the reverse transcriptase stage when the virus is not completely suppressed under the particular treatment conditions. This could be as a result of poor adherence, but also, as there are no current treatment regimens that are completely effective in halting viral replication, the emergence of drug-resistant HIV variants is a common occurrence.

Transmitted Drug Resistance (TDR), identified in patients recently infected and drug naive, suggests their infection was likely to have been acquired through contact with an individual already aware of their infection and undergoing HIV treatment. These data can be used to highlight current high risk behaviours among persons living with HIV. Studies in North America and Europe, investigating TDR in recently infected persons, reported the prevalence to be between 8 and 27% [439–450]. Nucleoside reverse transcriptase inhibitors (NRTI) resistance was consistently the most common mutation reported in newly infected patients [439–443, 445]. However, increase resistance rate against non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI) have also been identified [441, 443, 445].

Kiev City

Of the 118 persons for whom an HIV sequence was available (as of September 2014), 3 (2.5%) had evidence of TDR according to the Stanford algorithm []. All three were women classified as long-standing. Table 7.14 indicates the mutations identified, both NNRTI mutations result in high level resistance to Nevirapine (NVP) and Efavirenz (EFV). The NRTI mutations L74V and M184V are the most common among person's receiving Lumiviudine (3TC) and Emtricitabine (FTC), with L74V resulting in high level resistance to didanosine (ddI), and M184V, 3TC and FTC. the mutation K219E reduces susceptibility to Zidovudine (AZT).

Sample	PI	NRTI	NNRTI
1			G190S
2	L10I	L74V, M184V	K101A/E/P/Q, G190S
3		K219E	

Table 7.14: Resistant mutations identified among persons newly diagnosed with HIV between March 2013 and April 2014 in Kiev City.

7.4 Discussion

Residual samples from persons described in chapter 6 were tested with a TRI to determine the distribution of persons recently infected, and using key components (see Chapter 6) I estimated HIV incidence for Kiev City and Estonia. Persons newly diagnosed comprised all persons attending a VCT clinic across Poland, persons presenting for an HIV test at four testing sites in Kiev City and across all testing facilities within Estonia.

Between April 2013 and March 2014, HIV incidence for Kiev City was estimated at 21.5 new infections per 100,000 population, resulting in an estimate of 466 new infections. The distribution of those recently infected by risk further demonstrates the disproportionate distribution of HIV among MSM, with a third classified as recently infected. Using the same population estimates as described in section 5.8, incidence was estimated to be between 2289 and 6868 new infections per 100,000 MSM and 350 for PWID. These findings are similar to those reported for the USA using the same methodology, where the overall incidence estimate was 19.0, with over half of recent infections attributable to MSM.

The incidence rate of 21.5 was estimated using the modified methodology as described in section 4.3.2, where the probability of testing and being classified as recent used to extrapolate the true number of recent infections from the observed number of recent infections is estimated directly. The direct method of estimating this probability ensures the probability of testing and being classified as recent is not underestimated and therefore overestimating the number of new infections. This underestimation was identified as part of the original method by Karon *et al* where the inter-test interval is limited to 1 year and this probability is also bounded by the probability of being classified as recent. As a result, the original method overestimates incidence, with 25.9 per 100,000, resulting in an estimated 562 new infections. This original methodology has also been used to estimate incidence for France with 17.0 per 100,000 and 19.9 for the Italian region of Lazio, which although appear similar to that of with Kiev City, are likely to be lower if the same methodology was used.

The incidence estimate of 21.5 was extrapolated from persons where a recent infection was classified using RITA, where 6.2% of persons newly diagnosed at four testing sites in Kiev City were recently-infected. Initial classification of recent infections before correcting for

misclassified results was 8.4%, as 10 persons had a viral load <1000 copies/ml at diagnosis which has been identified as factor for a long-standing infection being classified as recent. Current work by CEPHIA evaluating TRIs indicated that treatment and a low viral load resulted in a high proportion of false recent infections [Gary Murphy, personal communication, March, 2013, [451]], rather than a low CD4 or AIDS diagnosis as previously thought. This change in methodology makes it difficult to compare findings already published, where recent infections identified among persons newly diagnosed correcting for CD4 and AIDS at diagnosis ranged from 11% in the UK to 35% in Australia. Alongside correcting for misclassified results, it is important to correct for longstanding infections that appear recent because a person may maintain an optical density below the assay's threshold for long periods following seroconversion. Within Kiev City, the FRR was estimated at 3.7%, higher than the suggested 2% by CEPHIA [Gary Murphy, personal communication, March, 2013]. However this is closer to the 2.7% FRR CEPHIA estimated for person with subtype A1 [Gary Murphy, personal communication, March, 2013], which is prominent in Ukraine.

Probable route of infection was found to be the only independent risk factor for testing as recent, with MSM at higher odds of testing recent compared to persons reporting heterosexual contact. Data on probable route of exposure is not extensively collected in Ukraine, which makes targeting at risk populations in terms of awareness, prevention and testing difficult. This disproportionate distribution of HIV among MSM indicates that onward transmission in this group is high, and highlights the need for tailored prevention and intervention strategies. Furthermore, the lack of risk information across Ukraine means that the true scale of the problem among these high risk groups is unknown.

Preliminary estimates for Estonia suggest the incidence rate among those self-initiating testing during 2013 was 95 per 100,000 population, considerably higher than estimates in Europe and the USA. The 1023 recent infections estimated to have occurred during 2013 were extrapolated from the 95 classified as recent using the LAg avidity EIA. Data on viral load were not available to correct for misclassified results, and with nearly half (44%) of persons diagnosed classified as recent we can assume this is an overestimation. In addition to correcting for viral load, a further factor for consideration is the recombinant form CRF06_cpx which is the predominate subtype across Estonia. It is unknown how the LAg Avidity EIA reacts with this recombinant form and

whether this interaction produces an artificially high proportion of recent infections. Further suggestions that this proportion is an overestimation is the high proportion of persons known to be longstanding but are classified as recent. Even after correcting for viral load, the FRR for Estonia was 5.9% considerably higher than the suggested 2% target. However, even though there is uncertainty in the number of those classified as recent, all persons with laboratory evidence of a negative test within 1 year were classified as recent, as were those reporting a seroconversion illness.

Initial results for Poland before correcting for viral load suggest the proportion of recent HIV infections in Poland was 30%. Although, I have not been able to correct for misclassified results, previous work in Poland showed that when using multiple TRI the proportion classified as recent fell from 47% to 22%. This high proportion of recent infections is likely to reflect the population using VCT sites for testing, with the majority of new diagnoses among MSM. As discussed previously MSM have been found to contribute a high proportion of recent infections compared with other risk groups. For France, Kiev City and the USA, half of recent infections were among MSM.

I also analysed previous work in Odessa using the BED-CEIA, indicating that approximately one in four persons with a confirmed new HIV diagnosis between May and December 2009, were recently-infected. This proportion is considerably higher than that found in Kiev City. Such a difference in part results from the limitations of the earlier study, with no data available to address misclassification of long-standing infections. Furthermore, the assay used was the BED-CEIA, which has been found to have a higher false-recent than the LAg Avidity EIA, with incidence estimates using the LAg Avidity assay much closer to modelled estimates than when the BED-CEIA was used [451]. Data from Odessa indicated that age was an independent risk factor for testing as recent, with a higher probability with decreasing age, consistent with findings from studies in the UK, France and Germany [378, 452, 453]. I also found evidence to suggest that women resident in the city and rural Odessa were more likely to test recent than their male counterparts. These findings may reflect the change in the current epidemic with the predominant route of infection in the Ukraine shifting from injecting drug use to heterosexual contact [1, 23], which was also evident in Kiev City (section 6.1.1).

Finally, as of September 2014 genotypic data were available for 25% persons newly diagnosed at the four testing sites in Kiev City suggesting a low level of transmitted drug resistance, considerably lower than published figures [440, 441, 445, 449]. Mutations identified were linked to NRTI's and NNRTI's, with two out of the three individuals with resistance to Nevirapine (NVP) and Efavirenz (EFV) both commonly used in first-line treatment and preventing mother to child transmission (PMTCT) suggesting that these persons were not newly diagnosed. This low-level TDR maybe because treatment coverage is low across Ukraine, estimated to be between 22 and 28%, rather than low onward transmission among persons who know their status.

Using the same data, I investigated the circulating subtype among those newly diagnosed within Kiev City. Consistent with published literature the majority were subtype A1. This subtype distribution is commonly identified among Eastern European countries and in particular is linked to PWID [454, 455]. The alternative MDRI as indicated by CEPHIA [Gary Murphy, personal communication, March, 2013] would reduce the incidence estimate for Kiev City. This signifys how the incidence rate is impacted by the probability of being classified as recent when using the initial methodology by Karon et al [409], and the difficulty in establishing the true number of recent infections.

Chapter 8

Discussion

This chapter summarises the main findings of the study, discusses how these outcomes contribute to research and compare with current literature. I identify limitations to the study and what further work is needed, and think about implications for policy and practice.

To better understand the spread of HIV in three Eastern European countries, Poland, Estonia, and Ukraine, a serological test for recent infection (TRI) was introduced to existing country surveillance to differentiate recent from non-recent infections. By identifying those who test close to their infection I was able to identify those at current risk of HIV and, for the first time, estimate HIV incidence for Estonia and the Ukrainian region of Kiev City.

Alongside the introduction of a TRI, laboratory, demographic, and clinical information was required to enable the population to be characterised and to correct for overestimation of recent infections. In Poland and Estonia, HIV surveillance is more developed but data collection within Ukraine consisted of basic information on the person's year of birth, sex, area of residence, and reason for test. Therefore, in addition to introducing a TRI within Ukraine, I successfully introduced a anonymous method of data collection, where persons would feel comfortable disclosing their risk factor information, with >99% acceptance rate. The collection of such information, through an anonymous questionnaire, enabled populations at risk to be identified, which has significant implications for policy, guidelines and funding.

HIV incidence for Kiev City (21.5 per 100,000 population) was similar to that reported for the USA using the same methodology [346], and significantly lower than estimates in Africa, where overall for Tanzania, Zimbabwe, and the South African regions of Soweto and Vulindlela incidence, was 1600 per 100,000 population [344]. Although incidence estimates were lower than expected, the diagnosis rate for Kiev City (21.5 per 100,000 population) was higher than the majority of countries within Europe, with only the overall rates for Ukraine and Estonia being higher at 37.1 and 23.5 per 100,000 population, respectively. Both new diagnoses and recent infections indicated a disproportionate distribution of HIV among MSM and PWID. Current data collection methods within Ukraine make it difficult to determine the distribution of persons at risk of HIV, with <1% diagnosed in 2012 known to be MSM [15]. Among those diagnosed in Kiev City, the data collected through this study suggest this proportion of MSM is higher (10%). If the proportion of MSM diagnosed overall in Ukraine was the same as Kiev City then the number of diagnoses increases from 152 to 1687 for 2013.

These results indicate the importance of a comprehensive surveillance system providing an accurate picture of those at greatest risk of HIV. Recommendations from ECDC suggest that

interventions should be relevant to national and local epidemiology, with surveillance data for Eastern Europe indicating that interventions for prevention and treatment should focus on PWID and their sexual partners [15].As described previously, the surveillance data for Ukraine is minimal with risk ascertained through reason for test. However, from the data collected within Kiev City, this would result in a key population being missed, a population where the majority of recent infections were identified.

Using published estimates to define the population at risk with Ukraine, incidence estimates for MSM ranged between 2289 new infections per 100,000 MSM based on 0.9% of the male population being MSM and 6868 if this proportion was 0.3%. These alarmingly high incidence rates compared with those for the overall population are similar to France [349] (1006 among MSM vs. 17 among the total population) and Los Angeles county [334] (493 vs. 23 respectively).

Work in the UK on the likely sources of onward transmission in this subpopulation suggests that high incidence among MSM is driven by condomless sex and would be even higher without the introduction of antiretroviral therapy [113]. ART coverage in Ukraine is one of the lowest in the world (between 22 and 28%) [456] and EMIS indicated that MSM in Ukraine are not being reached by prevention efforts, and have minimal understanding of HIV and its prevention [435]. This could be the result of MSM being less likely to identify as such because of stigma and discrimination but also due to the inadequate surveillance within Ukraine. Not understanding the risk factor distribution of HIV positive individuals makes appropriate and targeted public health interventions problematic. As there was little difference in the diagnosis rate between heterosexual men and women suggests non-disclosure among MSM in Kiev City was minimal; however it is difficult to determine how the population within the capital city reflects that of other regions in Ukraine.

Incidence estimates for PWID was also significantly higher than that for Kiev City overall. Like MSM, PWID are a hard to reach population, with underlying tension between law enforcements and those involved in public health [457]. Diagnosis figures for Ukraine (section 1.2) show sexual contact between men and women exceed that of injecting drug use to become the leading route of infection; however incidence estimates suggest that PWID are still an important subpopulation. UNAIDS indicates that harm reduction programmes are available in all regions

of Ukraine, but reaches only a third of those at risk, and <10% are accessing treatment [458]. Work modelling the effectiveness of medication-assisted treatment for opioid dependence (MAT), ART and syringe exchange programmes (SEP) among PWID, estimated that as high as 40% of HIV among PWID could have been avoided [37]. What has also become evident is a bridging between those who inject drugs and their sexual partners (section 6.1.1), with a much higher proportion of heterosexual women reporting an injecting partner compared to men.

Preliminary results in Estonia suggest an incidence rate of 95 per 100,000 population, although data were not yet available to correct for misclassification. These results are considerably higher than the incidence rate for Kiev before correcting for viral load, even though these countries have similar diagnosis rates. Of particular interest is how the LAg Avidity assay interacts with the recombinant subtype CRF06_cpx. The proportion of incorrectly classified recent infections according to the LAg avidity assay among persons known to be long-standing after correcting for viral load was considerably higher than what has been previously identified [Gary Murphy, personal communication, August, 2014], suggesting the assay performs differently with the CRF06_cpx subtype.

Estonia has a very similar HIV picture to Ukraine, with one of the highest rates of diagnosed HIV in Europe, where national surveillance suggests those at risk of HIV are predominately PWID or heterosexuals. The diagnosis rate for those tested during 2013 was consistent with that reported for 2012 (24.7 vs. 23.5 per 100,000 population), with the majority of those reporting a risk reporting heterosexual contact. With a similar distribution of sex, with 3 in 5 diagnosed among men, it is interesting to note that the proportion of diagnoses among men identified as MSM is much lower in Estonia than in Kiev City (6.7% vs. 15% respectively), with 6 men reporting their risk as MSM compared to 46 through the anonymous questionnaire in Kiev. It is possible there is non-disclosure of MSM with just over a third of diagnoses in Estonia with unknown risk, of which two thirds were men. However, unlike Kiev City where the majority of recent infections were identified among MSM, the distribution of recent infections appears to be similar across all risk groups. Although these results have not been corrected with viral load to give the true distribution of those with an observed recent infection the distribution is similar to Kiev City with a higher proportion among younger individuals and with the majority of recent infections among those with a negative test within 2 years of their initial diagnosis.

In Poland, preliminary results suggest the proportion recently infected was 30%, although viral load were not available for correction purposes. Whereas Estonia and Ukraine have the highest diagnosis rates in Europe, Poland has one of the lowest (2.8 per 100,000) at 1.2 for VCT attendees. The high proportion of recent infections is likely to reflect the distribution of those newly diagnosed, with the majority of new diagnoses among MSM. For Kiev City, France and the USA, over half of the recent infections identified were attributable to MSM [346, 349]. Incidence estimates were not available for Poland, the probability of testing and being classified as recent for first-time testers not currently available.

8.1 Limitations

There are several limitations worthy of discussion. Firstly, the data required to estimate the probability of testing and being classified as recent are prone to error. For repeat testers this is based on the time between the last negative test and their diagnostic test, which is therefore, reliant on these data to be reported. In Kiev City, as is likely in most surveillance systems, the availability of these data is based on self-reporting. In Estonia, on the other hand, where past HIV testing was linked, all persons with a negative test within a year were all classified as recent, but none of those self-reporting a negative test within a year. This suggests that relying on self-reporting of a previous negative result will increase the probability of testing and being classified as recent for repeat testers, and lead to an underestimate of the true number of recent infections. For first-time testers, the proportion of the population diagnosed late is used to estimate the time between infection and presenting for a test. For both Kiev City and Estonia, I used estimates from previous years and estimated the proportion among all new diagnoses rather than for first-time testers specifically. Ideally this probability should have been estimated for the population presenting for a test as part of the study. In Europe on average, where CD4 completion was >75%, 30% of persons were diagnosed with a CD4 cell count <200 cells/mm^3, with the average in the EU/EEA 26% similar to that estimated for Estonia, whereas among Non-EU/EEA countries this proportion was much higher at 43%, again similar to Kiev City.

Secondly, population data particularly for MSM within Kiev City was estimated using published literature, based on the work of AIDS Alliance and the European MSM internet survey (EMIS).

There are uncertainties within these estimates, indicated by their wide ranges, and further work is needed to establish the likely numbers at risk.

Thirdly, it is important to bear in mind that data from Kiev City may well not be generalizable to the rest of Ukraine. For a better understanding of the epidemic in other regions our methods need to be implemented across Ukraine. Acceptance rate was high (>99%) among attenders and information on HIV risk behaviour was also available for >99% of those completing the questionnaire.

Fourthly, although the LAg avidity was part of the evaluation process by CEPHIA, there are still uncertainties regarding how the assay performs among the recombinant subtype found in Estonia. Results among those known to be long-standing even after correcting for misclassification were considerably higher than previously identified suggesting recent infections are being over-represented.

Finally, for Kiev City, the proportion of persons diagnosed late was not available by subpopulations when extrapolating the true number of recent infections from the observed. Among populations where the proportion diagnosed late is likely to be higher than that for the overall population, the true number of recent infections is likely to be an overestimate and where it is low this is likely to be an underestimate.

8.2 Further Work

Recommendations for further work include national implementation of the new data collection method introduced in Kiev City. As data from Kiev City may not be representative of other regions, wider implementation would help ensure HIV prevention efforts and testing strategies are tailored to each region.

Efforts are currently underway to implement a more timely and centralised data collection facility within Poland. In addition, further work is required to understand what the barriers for testing are, and to increase testing frequencies, particularly in high risk populations, in line with testing guidelines. One of the main issues arising from this work is the inadequate levels of

testing. Through using the probability of testing and being classified as recent the true number of recent infections within the population is extrapolated from the number testing, which points to an undiagnosed population currently participating in risky behaviour and at risk of onward transmission.

Furthermore, there is a need to quantify the link from testing to care, particularly among persons recently infected who increase the risk of onward transmission. This will also aid the compilation of the cascade in the country.

Finally, understanding how the LAg avidity assay reacts with recommbinent subtypes such as the CRF06_cpx within Estonia is essential.

8.3 Implications for policy and practice

This work has several implications for policy and practice: the importance of diagnosing persons recently-infected; targeting the undiagnosed population; and recognising and targeting those at greatest risk of infection.

Recently infected individuals may be more likely to engage in high risk behaviours and contributes substantially to onward transmission of HIV. There is a need to diagnoses and target recently-infected persons. This is practically achieved by understanding of HIV and its prevention, and ensuring frequent testing within at-risk groups. Furthermore, the extrapolation process from the observed to the true number of recent infections using the testing frequencies of the population represents the high numbers of undiagnosed infections within sub-population. This requires targeted testing, increasing knowledge of the importance of frequent testing, and ensuring ease of access to testing facilities.

Although testing facilities are being accessed, the limited data currently collected in Ukraine means that prevention and intervention efforts may not be relevant to the local epidemiology. Inadequate surveillance and risk behaviour ascertainment results in a lack of prioritisation and support among at-risk groups. Lack of knowledge regarding the sub-populations at greatest risk makes it increasingly difficult to target in terms of increasing awareness, prevention, and

promoting testing.

Finally, efforts need to be made to increase referral into care and treatment coverage. In Ukraine, treatment coverage and access to HIV-related care is particularly poor, during 2012, only 67% of persons newly diagnosed in Ukraine accessed HIV related care following diagnosis, with the estimated ART coverage of 28% [459].

8.4 Conclusions

This work has shown the importance of having a better understanding of the epidemic to ensure interventions for prevention, and promotion of testing services are tailored to key populations. Within Kiev City this not only meant understanding current transmission patterns using a TRI, but for detailed surveillance information to have been collected. This knowledge base will better enable targeted public health action, health promotion work, laying a foundation to enable local and national guidelines to be developed, and to support work being conducted by government and non-government organisations.

This study speaks to the need for targeted testing. The extrapolation process to estimate the true number of recent infections indicates those populations are currently undiagnosed participating in high-risk behaviour and at risk of onward transmission. Further work is needed to understand what the barriers for testing are, and to increase testing frequencies, particularly in high risk populations, in line with testing guidelines.

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Appendix A

Incidence estimates

Incidence estimates from published studies identified using the method in section 2.2. Publications grouped by study region, method of estimating incidence and population of focus All incidence estimates were present by 100 pyrs at risk (or of follow-up).

High risk popualtions included: Commercial Sex Workers, Discordant Couples, High risk adults, Injecting Drug Users, Men who have sex with men, persons of transgender, Prisoners and Sero-discordant Married Couples. Testing populations included: Anonymous Testing, General Testing Population, STD Clinic, Voluntary counselling and testing, Vaccine Cohorts, Injecting drug use programs, New diagnoses.Other included: Accident and Emergency, Antenatal, Blood Donors, Heterosexual contact, Community-based Cohorts, Male factory Workers, Household-Based Surveillance, Military, Married Couples, Police Officers, Population based survey.

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Africa	Mombasa, Kenya	1993-1997	High Risk	13.1		cohort	Prospective	Baeten	2000	[244]
Africa	South Africa	2005-2006	Other	3.03	2.44-3.63	RITA	BED-CEIA McDougal	Barnighausen	2008	[125]
Africa	South Africa	2005-2006	Other	3.17	2.54-3.80	RITA	BED-CEIA McWalter/Welte	Barnighausen	2008	
Africa	South Africa	2005-2006	Other	3.22	2.57-3.87	RITA	BED-CEIA McWalter/Welte	Barnighausen	2008	
Africa	South Africa	2005-2006	Other	3.19	2.57-3.82	RITA	BED-CEIA Hargrove	Barnighausen	2008	
Africa	South Africa	2005-2006	Other	3.17	2.54-3.81	RITA	BED-CEIA McWalter/Welte	Barnighausen	2008	
Africa	South Africa	2003-2006	Other	2.91	2.56-3.32	cohort	Prospective	Barnighausen	2008	
Africa	KwaZulu-Natal	2003-2005	Other	3.8		cohort	Prospective	Barnighausen	2008	[243]
Africa	KwaZulu-Natal	2003-2005	Other	2.3		cohort	Prospective	Barnighausen	2008	
Africa	Kinshasa	1986-1989	Other	2.8	1.4-4.2	Other	Serial Seroprevalence	Batter	1994	[460]
Africa	Uganda	1989-2007	Other	7.11		cohort	Retrospective	Biraro	2013	[269]
Africa	Rwanda		High Risk	12.2	8.2-16.2	RITA	BED-CEIA	Braunstein	2010	[240]
Africa	Rwanda		High Risk	9.8	6.6-13.0	RITA	BED-CEIA Hargrove	Braunstein	2010	
Africa	Rwanda		High Risk	7.4	4.4-10.4	RITA	BED-CEIA-Avidity	Braunstein	2010	
Africa	Rwanda		High Risk	5.1	2.8-7.5	RITA	BED-CEIA-Avidity excluding CD4 <500	Braunstein	2010	
Africa	Rwanda		High Risk	15.7	11.6-19.8	RITA	Avidity	Braunstein	2010	
Africa	Rwanda		High Risk	11.1	8.2-41.0	RITA	Avidity Hargrove	Braunstein	2010	
Africa	Rwanda		High Risk	3.5	1.6-3.32	cohort	Prospective	Braunstein	2010	
Africa	Rwanda		High Risk	7.2	4.0-10.3	RITA	BED-CEIA excluding CD4 <500	Braunstein	2010	
Africa	Rwanda		High Risk	5.9	3.5-8.3	RITA	BED-CEIA-Avidity Hargrove	Braunstein	2010	
Africa	Rwanda		High Risk	8.2	5.1-11.4	RITA	Avidity excluding CD4 <500	Braunstein	2010	
Africa	Rwanda	1989-1993	Other	2.7	1.8-3.9	cohort	Prospective	Bulterys	2004	[238]
Africa	Rwanda	1989-1993	Other	5.7	4.9-5.5	RITA	BED-CEIA	Bulterys	2004	
Africa	Ethiopia		High Risk	2.6	1.4-3.8	RITA	BED-CEIA Welte	Combes	2013	[316]
Africa	Ethiopia		High Risk	3.3	2.1-4.5	RITA	BED-CEIA	Combes	2013	
Africa	Ethiopia		High Risk	2.7	1.7-3.7	RITA	BED-CEIA Hargrove	Combes	2013	
Africa	Zimbabwe	2002-2004	Testing population	1.21	0.92-1.64	cohort	Prospective	Corbett	2007	[237]
Africa	Bloemfontein-RSA	2009	High Risk	5.5	2.5-10.4	cohort	Prospective	Feldblum	2012	[230]
Africa	Rustenburg-RSA	2008-2009	High Risk	3	0.4-10.8	cohort	Prospective	Feldblum	2012	
Africa	Hlabisa-South Africa	1999	Other	17.9		RITA	Detuned	Gouws	2002	[282]
Africa	Hlabisa-South Africa	1999	Other	14.4		Other	Age specific model	Gouws	2002	
Africa	Zimbabwe	1993-1995	Other	2.02		cohort	Prospective	Gregson	1998	[229]
Africa	Zimbabwe	1993-1995	Other	2.07		Other	Cumulative incidence and survival	Gregson	1998	
Africa	Zimbabwe	1993-1995	Other	1.98		Other	Constant Prevalence Methods	Gregson	1998	
Africa	Zimbabwe	1997-2000	Other	5.5	5.0-6.0	RITA	BED-CEIA McDougal	Hargrove	2008	[320]
Africa	Zimbabwe	1997-2000	Other	9.5	8.7-10.3	RITA	BED-CEIA	Hargrove	2008	
Africa	Zimbabwe	1997-2000	Other	3.5	2.6-4.5	RITA	BED-CEIA Hargrove	Hargrove	2008	
Africa	Zimbabwe	1997-2000	Other	3.5	2.9-4.2	RITA	BED-CEIA	Hargrove	2008	
Africa	Zimbabwe	1997-2000	Other	6	5.2-6.9	RITA	BED-CEIA Hargrove	Hargrove	2008	
Africa	Zimbabwe	1997-2000	Other	7.6	6.7-8.5	RITA	BED-CEIA including LS	Hargrove	2008	
Africa	South Africa	1999-2001	Other	0.45		RITA	Detuned	Heyns	2002	[285]
Africa	Uganda	1996	Other	4.6		cohort	Prospective	Kamali	2000	[220]
Africa	Uganda	1995	Other	6.2		cohort	Prospective	Kamali	2000	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Africa	Uganda	1994	Other	6		cohort	Prospective	Kamali	2000	
Africa	Uganda	1993	Other	9.5		cohort	Prospective	Kamali	2000	
Africa	Uganda	1992	Other	6.1		cohort	Prospective	Kamali	2000	
Africa	Uganda	1991	Other	6.3		cohort	Prospective	Kamali	2000	
Africa	Uganda	1990	Other	7.7		cohort	Prospective	Kamali	2000	
Africa	KwaZulu-Natal	2004-2007	Testing Population	6.4	2.6-13.2	cohort	Prospective	Karim	2011	[219]
Africa	KwaZulu-Natal	2004-2007	Other	6.5	4.4-9.2	cohort	Prospective	Karim	2011	
Africa	Kakira	2004	High Risk	5.2		RITA	BED-CEIA McDougal	Karita	2007	[323]
Africa	Masaka	2004	High Risk	6.4		RITA	BED-CEIA McDougal	Karita	2007	
Africa	Kilifi-Kenya	2004	High Risk	2.5		RITA	BED-CEIA McDougal	Karita	2007	
Africa	Kangemi	2004	High Risk	1.7		RITA	BED-CEIA McDougal	Karita	2007	
Africa	Masaka	2004	High Risk	6.8		RITA	BED-CEIA Hargrove	Karita	2007	
Africa	Kangemi	2004	High Risk	3.4	1.5-5.3	RITA	BED-CEIA	Karita	2007	
Africa	Kilifi-Kenya	2004	High Risk	3.5	2.1-4.9	RITA	BED-CEIA	Karita	2007	
Africa	Kakira	2004	High Risk	6	4.3-7.7	RITA	BED-CEIA	Karita	2007	
Africa	Masaka	2004	High Risk	6.1	4.2-8.0	RITA	BED-CEIA	Karita	2007	
Africa	Kangemi	2004	High Risk	1.9		RITA	BED-CEIA Hargrove	Karita	2007	
Africa	Kilifi-Kenya	2004	High Risk	2.7		RITA	BED-CEIA Hargrove	Karita	2007	
Africa	Kakira	2004	High Risk	5.6		RITA	BED-CEIA Hargrove	Karita	2007	
Africa	Malawi	2009	Other	4	2.2-7.2	cohort	Prospective	Keating	2012	[218]
Africa	Meru, Kenya		High Risk	1.8		RITA	Avidity	Khamadi	2009	[273]
Africa	Thika, Kenya		High Risk	5.67		RITA	BED-CEIA	Khamadi	2009	
Africa	Nandi, Kenya		High Risk	6.72		RITA	BED-CEIA	Khamadi	2009	
Africa	Naivasha, Kenya		High Risk	2.86		RITA	BED-CEIA	Khamadi	2009	
Africa	Meru, Kenya		High Risk	3.91		RITA	BED-CEIA	Khamadi	2009	
Africa	Thika, Kenya		High Risk	3.2		RITA	Avidity	Khamadi	2009	
Africa	Naivasha, Kenya		High Risk	2.1		RITA	Avidity	Khamadi	2009	
Africa	Nandi, Kenya		High Risk	4.1		RITA	Avidity	Khamadi	2009	
Africa	South Africa	2005	Other	2.00	1.7-2.4	Other	EPP and Spectrum modeled	Kim	2010	[324]
Africa	South Africa	2005	Other	4.4	2.3-6.5	RITA	BED-CEIA	Kim	2010	
Africa	South Africa	2005	Other	2.40	1.7-3.1	RITA	BED-CEIA Hargrove	Kim	2010	
Africa	Cote dÃ¦Ivoire	2004	Other	2.9	2.1-3.7	RITA	BED-CEIA Hargrove	Kim	2010	
Africa	Cote dÃ¦Ivoire	2004	Other	3.8	3.3-4.5	RITA	BED-CEIA	Kim	2010	
Africa	Kenya	2003	Other	2.6	2.0-3.2	RITA	BED-CEIA Hargrove	Kim	2010	
Africa	Kenya	2003	Other	1	1.02-1.08	Other	EPP and Spectrum modeled	Kim	2010	
Africa	Kenya	2003	Other	3.5	2.7-4.3	RITA	BED-CEIA	Kim	2010	
Africa	Cote dÃ¦Ivoire	2002	Other	4.5	3.0-5.9	RITA	BED-CEIA	Kim	2010	
Africa	Cote dÃ¦Ivoire	2002	Other	3.5	2.3-4.6	RITA	BED-CEIA Hargrove	Kim	2010	
Africa	Cote dÃ¦Ivoire	2000	Other	1.9	1.3-2.5	RITA	BED-CEIA Hargrove	Kim	2010	
Africa	Cote dÃ¦Ivoire	2000	Other	2.9	2.0-3.9	RITA	BED-CEIA	Kim	2010	
Africa	Cote dÃ¦Ivoire	1998	Other	3.6	2.6-4.5	RITA	BED-CEIA Hargrove	Kim	2010	
Africa	Cote dÃ¦Ivoire	1998	Other	4.6	3.4-5.8	RITA	BED-CEIA	Kim	2010	
Africa	Malawi	1998	Other	3.1	2.1-4.5	cohort	Prospective	Kumwenda	2001	[211]

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Africa	Malawi	1994	Other	10.9	9.4-12.6	cohort	Prospective	Kumwenda	2001	
Africa	Tanzania	2009-2011	Other	0.78		RITA	MAA	Laeyendecker	2013	[344]
Africa	Soweto, South Africa	2009-2011	Other	1.18		RITA	MAA	Laeyendecker	2013	
Africa	Vulindlela, South Africa	2009-2011	Other	3.9		RITA	MAA	Laeyendecker	2013	
Africa	Overall	2009-2011	Other	1.6		RITA	MAA	Laeyendecker	2013	
Africa	Zimbabwe	2009-2011	Other	0.91		RITA	MAA	Laeyendecker	2013	
Africa	Zimbabwe	1998-2000	Other	1.99	1.63-2.42	cohort	Prospective	Lopman	2008	[206]
Africa	Zimbabwe	1998-2000	Other	1.57	1.30-08.9	cohort	Prospective	Lopman	2008	
Africa	Uganda		Other	1.6		cohort	Prospective	Matovu	2006	[205]
Africa	Harare, Zimbabwe	1993-1995	Other	2.93	2.18-3.86	cohort	Prospective	Mbizvo	1996	[204]
Africa	Uganda	1990-1999	Other	0.59	0.52-0.68	cohort	Prospective	Mbulaiteye	2002	[203]
Africa	Uganda	2004-2005	Other	1.8	1.5-2.1	RITA	BED-CEIA	Mermin	2008	[328]
Africa	Der es Salaam, Tanzania	2005-2008	Other	0.84	0.468-1.403	cohort	Prospective	Munseri	2013	[196]
Africa	KwaZulu-Natal, Edendale	2007-2009	Other	6.3	3.2-9.4	cohort	prospective	Nel	2012	[195]
Africa	KwaZulu-Natal, Ladysmith	2007-2009	Other	14.8	9.7-19.8	cohort	prospective	Nel	2012	
Africa	KwaZulu-Natal, Pinetown	2007-2009	Other	7.2	3.7-10.7	cohort	prospective	Nel	2012	
Africa	Kenya	2002-2003	Testing population	1.3	0.87-1.73	RITA	Detuned	Oyugi	2004	[296]
Africa	Cape Town-South Africa	2005-2006	High Risk	2.0	1.0-4.1	cohort	Prospective	Price	2012	[192]
Africa	Nairobi-Kenya	2005-2006	High Risk	1.3	0.7-2.2	cohort	Prospective	Price	2012	
Africa	Kilifi-Kenya	2005-2006	High Risk	3.5	2.6-4.7	cohort	Prospective	Price	2012	
Africa	Kenya	2008	High Risk	5.6	1.62-11.67	cohort	Prospective	Priddy	2011	[191]
Africa	South Africa	2005-2008	Other	1.3	0.6-2.1	Other	Two Cross-sectional Prevalence Measurements	Rehle	2010	[352]
Africa	South Africa	2005	Other	1.4	1.0-1.8	RITA	BED-CEIA McDougal	Rehle	2007	
Africa	South Africa	2005	Other	2.4	2.2 - 2.7	RITA	BED-CEIA McDougal	Rehle	2007	
Africa	South Africa	2002-2005	Other	2	1.2-3.0	Other	Two Cross-sectional Prevalence Measurements	Rehle	2010	
Africa	Tanzania	2000	Other	10	7.0-13.8	cohort	Prospective	Riedner	2006	[186]
Africa	Masaka, Uganda	2006-2009	High Risk	4.3	3.1-6.0	cohort	Prospective	Ruzagira	2011	[182]
Africa	Masaka, Uganda	2004-2007	Testing Population	1.04	0.68-1.59	cohort	Prospective	Ruzagira	2011	[183]
Africa	Bujumbura, Burundi	1990-1993	Other	1		Other	Model age specific incidence	Saidel	1996	[105]
Africa	Bujumbura, Burundi	1990-1993	Other	2.3		cohort	Prospective	Saidel	1996	
Africa	Bujumbura, Burundi	1990-1993	Other	2.7		Other	Model age specific incidence	Saidel	1996	
Africa	Bujumbura, Burundi	1990-1993	Other	1.5		cohort	Prospective	Saidel	1996	
Africa	Bujumbura, Burundi	1990-1993	Other	1.5		Other	Model age specific incidence	Saidel	1996	
Africa	Bujumbura, Burundi	1990-1993	Other	1.8		Other	Model age specific incidence	Saidel	1996	
Africa	Bujumbura, Burundi	1990-1993	Other	1.6		cohort	Prospective	Saidel	1996	
Africa	Bujumbura, Burundi	1990-1993	Other	1.8		cohort	Prospective	Saidel	1996	
Africa	Bujumbura, Burundi	1990-1993	Other	2.2		cohort	Prospective	Saidel	1996	
Africa	Bujumbura, Burundi	1990-1993	Other	2.0		Other	Model age specific incidence	Saidel	1996	
Africa	Tanzania	1991-1996	Other	1.92	1.22-2.89	cohort	Prospective	Senkoro	2000	[179]
Africa	Tanzania	1991-1996	Other	1.05	0.72-1.48	cohort	Prospective	Senkoro	2000	
Africa	Uganda	2004	Other	5.8		cohort	Prospective	Shafer	2008	[177]
Africa	Uganda	2004	Other	3.9		cohort	Prospective	Shafer	2008	
Africa	Uganda	1999	Other	5.7		cohort	Prospective	Shafer	2008	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Africa	Uganda	1999	Other	3.2		cohort	Prospective	Shafer	2008	
Africa	Uganda	1994	Other	10.2		cohort	Prospective	Shafer	2008	
Africa	Uganda	1994	Other	2.1		cohort	Prospective	Shafer	2008	
Africa	Uganda	1990	Other	5.9		cohort	Prospective	Shafer	2008	
Africa	Uganda	1990	Other	9.1		cohort	Prospective	Shafer	2008	
Africa	Kenya	2006	Other	1	0.77-1.28	cohort	Prospective	Shaffer	2010	[176]
Africa	Kenya	2005	Other	1.16	0.86-1.54	cohort	Prospective	Shaffer	2010	
Africa	Kenya	2004	Other	1.41	0.95-2.02	cohort	Prospective	Shaffer	2010	
Africa	Zimbabwe	2002-2003	Testing population	1.91	1.09-2.72	RITA	BED-CEIA	Troung	2011	[338]
Africa	Tanzania	1999-2004	Other	12.6	6.4-18.9	cohort	Prospective	Wambura	2007	[168]
Africa	Tanzania	1996-2000	Other	18.6	16.5-20.7	cohort	Prospective	Wambura	2007	
Africa	Tanzania	1994-1997	Other	11.2	8.2-14.2	cohort	Prospective	Wambura	2007	
Africa	Durban, South Africa	2003-2006	Other	6.8		cohort	Prospective	Wand	2012	[167]
Africa	Durban, South Africa	2002-2005	Other	6.6		cohort	Prospective	Wand	2011	[166]
Africa	Hlabisa, South Africa	1998	Other	11.4	10.0-13.1	Other	Age Prevalence Model	Williams	2001	[109]
Africa	Addis Ababa-Ethiopia	2003	Other	2.0	0.7-3.3	RITA	BED-CEIA	Xu	2010	[157]
Africa	Addis Ababa-Ethiopia	2002	Other	2.7	1.2-4.2	RITA	BED-CEIA	Xu	2010	
Africa	Addis Ababa-Ethiopia	2001	Other	5.4	3.3-7.6	RITA	BED-CEIA	Xu	2010	
Africa	Addis Ababa-Ethiopia	2000	Other	2.7	1.2-4.3	RITA	BED-CEIA	Xu	2010	
Africa	Addis Ababa-Ethiopia	1997	Other	5.3	3.1-7.5	RITA	BED-CEIA	Xu	2010	
Africa	Addis Ababa-Ethiopia	1996	Other	6.3	3.9-8.8	RITA	BED-CEIA	Xu	2010	
Africa	Addis Ababa-Ethiopia	1995	Other	7.7	3.9-11.5	RITA	BED-CEIA	Xu	2010	
Africa	Malawi	2003-2005	Other	4.51	2.96-6.06	Cohort	Prospective	Kumwendo	2008	[136]
Africa	Uganda	1990-1994	Population Survey	0.71	0.56-0.85	Cohort	Prospective	Kengeyon-kayondo	1996	[137]
Africa	Masaka, Uganda	1989-1990	Population Survey	0.92	0.55-1.29	Cohort	Prospective	Mulder	1994	[138]
Africa	Kigali, Rwanda	1988-1992	Other	3.5	1.9-5.0	Cohort	Prospective	Leroy	1994	[139]
Africa	Tanzania	2004-2008	Population Survey	0.34		Other	Serial Seroprevalence	Hallett	2010	[461]
Africa	Zambia	2002-2007	Population Survey	1.12		Other	Serial Seroprevalence	Hallett	2010	
Africa	Dominican Republic	2002-2007	Population Survey	0.05		Other	Serial Seroprevalence	Hallett	2010	
Africa	Niger	2002-2006	Population Survey	0.06		Other	Serial Seroprevalence	Hallett	2010	
Africa	Mali	2001-2006	Population Survey	0.11		Other	Serial Seroprevalence	Hallett	2010	
Africa	Malawi & Zimbabwe	1999-2001	Other	4.7	3.8-5.6	Cohort	Prospective	Kumwendo	2006	[135]
Africa	South Africa	2003-2007	Population Survey	3.4	3.1-5.7	Cohort	Prospective	Barnighausen	2009	[142]
Africa	Nigeria	2006	High Risk	12.36	8.18-16.34	RITA	BED-CEIA Welte	Forbi	2011	[313]
Africa	Nigeria	2006	High Risk	11.97	8.5-15.43	RITA	BED-CEIA Hargrove	Forbi	2011	
Africa	Durban, South Africa	2003-2005	Other	6.75	5.74-7.93	Cohort	Prospective	Mavedzenge	2011	[146]
Africa	Johannesburg, South Africa	2003-2005	Other	3.33	2.51-4.44	Cohort	Prospective	Mavedzenge	2011	
Africa	Havare, South Africa	2003-2005	Other	2.72	2.26-3.26	Cohort	Prospective	Mavedzenge	2011	
Africa	Kwazulu-Natal, South Africa	2002-2005	Other	6.6		Cohort	Prospective	Ramjee	2011	[150]
Africa	Windhock, Nambia	2007-2009	Population Survey	2.4	1.9-2.9	Cohort	Prospective	Anlagnier	2011	[141]
Americas	Brazil	1996-1999	Testing population	2	1.1-3.5	RITA	Detuned	Alves	2003	[275]
Americas	Argentina	2000-2001	High Risk	6.68	3.10-11.29	RITA	Detuned	Avila	2002	[245]
Americas	Argentina	2000-2001	High Risk	6	3.10-11.0	cohort	Prospective	Avila	2002	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Americas	USA	2008	High Risk	2.9		cohort	Retrospective	Balaji	2013	[270]
Americas	Brazil	1998-2001	Other	0.0269	0.0189-0.0349	RITA	Detuned	Barreto	2005	[276]
Americas	USA	1986-2005	Other	0.015	0.014-0.016	cohort	Prospective	Baustista	2006	[242]
Americas	Atlanta-Los Angeles-san Francisco	2004-2005	High Risk	9.5	3.9-15.1	RITA	BED-CEIA	Buchacz	2008	[315]
Americas	Atlanta-Los Angeles-san Francisco	2004-2005	High Risk	10.5	2.7-18.3	RITA	BED-CEIA	Buchacz	2008	
Americas	USA	1995-1997	High Risk	1.55	1.23-1.95	cohort	Prospective	Buchbinder	2005	[239]
Americas	Canada	2000	High Risk	1.16	0.79-1.53	cohort	Retrospective	Calzavara	2002	[268]
Americas	Canada	2000	High Risk	0.14	0.02-0.27	cohort	Retrospective	Calzavara	2002	
Americas	Canada	2000	High Risk	0.25	0.05-0.44	cohort	Retrospective	Calzavara	2002	
Americas	Canada	2000	Other	0.03	0.01-0.05	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1999	High Risk	0.10	0-0.19	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1999	High Risk	0.39	1.04-1.75	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1999	High Risk	0.28	0.13-0.43	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1999	Other	0.04	0.02-0.06	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1998	High Risk	0.98	0.70-1.27	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1998	High Risk	0.28	0.14-0.42	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1998	High Risk	0.08	0-0.16	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1998	Other	0.03	0.01-0.04	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1997	High Risk	0.21	0.09-0.33	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1997	High Risk	1.04	0.76-1.31	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1997	High Risk	0.09	0.01-0.16	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1997	Other	0.02	0.01-0.03	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1996	High Risk	0.79	0.56-1.03	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1996	High Risk	0.23	0.1-0.36	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1996	Other	0.02	0-0.03	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1996	High Risk	0.11	0.02-0.20	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1995	Other	0.03	0.01-0.04	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1995	High Risk	0.11	0.02-0.20	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1995	High Risk	0.95	0.69-1.22	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1995	High Risk	0.33	0.17-0.49	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1994	High Risk	0.59	0.36-0.82	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1994	High Risk	0.15	0.03-0.26	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1994	Other	0.03	0.01-0.05	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1994	High Risk	1.17	0.85-1.48	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1993	Other	0.03	0-0.05	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1993	High Risk	0.40	0.17-0.62	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1993	High Risk	0.08	0-0.18	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1993	High Risk	1.41	1.00-1.81	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1992	High Risk	0.01	0-0.08	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1992	Other	0.02	0-0.05	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1992	High Risk	0.64	0.18-1.10	cohort	Retrospective	Calzavara	2002	
Americas	Canada	1992	High Risk	1.23	0.63-1.82	cohort	Retrospective	Calzavara	2002	
Americas	USA	2000-2001	High Risk	1.8	0.3-6.5	RITA	Detuned	Choi	2004	[277]

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Americas	Trinidad	2001	Testing Population	4.2	4.1-4.5	RITA	Detuned	Cleghorn	2002	[278]
Americas	Trinidad	2000	Testing Population	2.2		RITA	Detuned	Cleghorn	2002	
Americas	Trinidad	1994-1997	Testing Population	6.9	4-11.2	cohort	Prospective	Cleghorn	1998	[68]
Americas	Trinidad	1993-1995	Testing Population	5	2.7-8.6	RITA	p24 antigen	Cleghorn	1998	
Americas	Trinidad	1987-1991	Testing Population	4.5	2.5-7.6	cohort	Retrospective	Cleghorn	1998	
Americas	Trinidad	1987-1991	Testing Population	3.5	2.9-4.1	Other	Prevalence data	Cleghorn	1998	
Americas	Vancouver	1996-2000	High Risk	12.7	10.-15.1	cohort	Prospective	Craib	2003	[236]
Americas	Brazil	2001-2002	Other	0.32	0.02-1.39	RITA	Detuned	De Freitas Olivera	2005	[279]
Americas	Brazil	1999-2002	Other	0.22	0.041-0.608	RITA	Detuned	De Freitas Olivera	2005	
Americas	Brazil	1999-2000	Other	0.17	0.01-0.64	RITA	Detuned	De Freitas Olivera	2005	
Americas	Brazil	2006-2009	Testing population	0.73	0.61-0.86	RITA	BED-CEIA	De Lima	2012	[317]
Americas	New York	1998-1999	High Risk	0.88	0.31-1.45	cohort	Prospective	Deren	2004	[233]
Americas	Puerto Rico	1998-1999	High Risk	3.37	2.02-4.72	cohort	Prospective	Deren	2004	
Americas	New York	1996-2002	High Risk	0.86	0.42-1.29	RITA	Detuned	Des Jarlais	2005	[280]
Americas	New York	1995-1997	Testing Population	1.35	0.645-2.473	cohort	Prospective	Des Jarlais	2000	[221]
Americas	New York	1995-1997	Testing Population	0.67	0.247-1.467	cohort	Prospective	Des Jarlais	2000	
Americas	New York	1994-1997	Testing Population	0.85	0.022-4.753	cohort	Prospective	Des Jarlais	2000	
Americas	New York	1993-1995	Testing Population	2.96	0.359-10.703	cohort	Retrospective	Des Jarlais	2000	
Americas	New York	1992-1995	Testing Population	1.58	0.326-4.627	cohort	Retrospective	Des Jarlais	2000	
Americas	New York	1992-1995	Testing Population	0	0.000-7.177	cohort	Prospective	Des Jarlais	2000	
Americas	New York	1992-1995	Testing Population	1.17	0.584-2.092	cohort	Prospective	Des Jarlais	2000	
Americas	New York	1991-1997	Testing Population	0.48	0.219-0.908	cohort	Prospective	Des Jarlais	2000	
Americas	New York	1990-1997	Testing Population	0.6	0.258-1.177	cohort	Retrospective	Des Jarlais	2000	
Americas	New York	1990-1995	High Risk	3.09	1.88-4.30	RITA	Detuned	Des Jarlais	2005	[280]
Americas	USA	2009-2010	High Risk	0.24	0.07-0.62	cohort	Prospective	Eshleman	2013	[231]
Americas	USA	2009-2010	High Risk	0.25	0.03-0.93	RITA	MAA (Enrolment)	Eshleman	2013	
Americas	USA	2009-2010	High Risk	1.36	0.091-5.02	RITA	RNA (26 day window)	Eshleman	2013	
Americas	USA	2009-2010	High Risk	0.13	0.006-0.076	RITA	MAA (End of Study)	Eshleman	2013	
Americas	USA	2009-2010	High Risk	2.52	1.7-9.33	RITA	RNA (14 day window)	Eshleman	2013	
Americas	USA	1988-1997	Testing population	1.3	0.7-7.0	cohort	Retrospective	Fernyak	2002	[271]
Americas	Sullana	2006	High Risk	1.4	0.0-3.0	RITA	BED-CEIA	Guanira	2007	[319]
Americas	Guayaquil	2006	High Risk	7.4	3.5-11.9	RITA	BED-CEIA	Guanira	2007	
Americas	Lima	2006	High Risk	3.1	1.0-5.3	RITA	BED-CEIA	Guanira	2007	
Americas	Ica	2006	High Risk	2.4	0.0-4.8	RITA	BED-CEIA	Guanira	2007	
Americas	Dominican Republic	2004-2005	High Risk	0.9	0.1-4.5	RITA	Avidity	Gupta	2007	[284]
Americas	Dominican republic	2004-2005	High Risk	1.4	0.2-5.3	RITA	Detuned	Gupta	2007	
Americas	Dominican Republic	2004-2005	High Risk	1	0.1-4.4	RITA	BED-CEIA	Gupta	2007	
Americas	USA	2006	Other	0.0228	0.0195-0.0261	RITA	BED-CEIA	Hall	2008	[345]
Americas	Brazil	1995-1997	High Risk	3.10	2.1-4.1	cohort	Prospective	Harrison	1999	[228]
Americas	USA	2009-2010	High Risk	0.32	0.14-0.74	cohort	Prospective	Hodder	2013	[226]
Americas	Brazil	2004	High Risk	4.99	0.051-34.7	RITA	Detuned	Hoffmann Pfrimer	2008	[286]
Americas	Brazil	2004	High Risk	0.83	0.009-5.6	RITA	Detuned	Hoffmann Pfrimer	2008	
Americas	Canada	2000	High Risk	1.4	0.8-1.9	cohort	Prospective	Hogg	2001	[225]

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Americas	New York	2007	High Risk	3.31	1.43-6.47	RITA	Detuned	Jenness	2011	[287]
Americas	san Francisco	1998	High Risk	2		cohort	Retrospective	Kellogg	1999	[262]
Americas	san Francisco	1997	High Risk	1.2		cohort	Retrospective	Kellogg	1999	
Americas	san Francisco	1996-2002	Other	0.036	0.0-0.1	RITA	Detuned	Kellogg	2005	[259]
Americas	san Francisco	1996-2002	High Risk	0.78	0.60-1.0	Cohort	Retrospective (Self-reported)	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	4.2	3.2-5.3	Cohort	Retrospective (Self-reported)	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	1.6	1.5-1.9	Cohort	Retrospective (Self-reported)	Kellogg	2005	
Americas	san Francisco	1996-2002	Testing population	1	0.96-1.1	Cohort	Retrospective (Self-reported)	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	0	0.0-1.1	RITA	Detuned	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	4.8	2.2-9.7	RITA	Detuned	Kellogg	2005	
Americas	san Francisco	1996-2002	Other	0.032	0.0-0.1	RITA	Detuned	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	5.5	0.0-17.8	RITA	Detuned	Kellogg	2005	
Americas	san Francisco	1996-2002	Other	2.4	1.5-3.3	RITA	Detuned	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	5.5	3.7-7.9	Cohort	Retrospective (Self-reported)	Kellogg	2005	
Americas	san Francisco	1996-2002	Other	0.57	0.1-2.7	cohort	Retrospective	Kellogg	2005	
Americas	san Francisco	1996-2002	Other	0.21	0.15-0.26	Cohort	Retrospective (Self-reported)	Kellogg	2005	
Americas	san Francisco	1996-2002	Testing population	1.4	0.8-2.0	RITA	Detuned Satten	Kellogg	2005	
Americas	san Francisco	1996-2002	Testing population	1.3	0.93-1.8	RITA	Detuned	Kellogg	2005	
Americas	san Francisco	1996-2002	Other	0.48	0.1-2.3	cohort	Retrospective	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	2	1.4-2.8	RITA	Detuned	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	2.6	0.1-12.5	cohort	Retrospective	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	1	0.17-3.1	cohort	Retrospective	Kellogg	2005	
Americas	san Francisco	1996-2002	High Risk	1.4	0.90-2.1	cohort	Retrospective	Kellogg	2005	
Americas	san Francisco	1996-2002	Testing population	1.2	0.80-1.6	cohort	Retrospective	Kellogg	2005	
Americas	san Francisco	1996-2002	Other	3	2.8-3.3	Cohort	Retrospective (Self-reported)	Kellogg	2005	
Americas	san Francisco	1996-2002	Other	0.27	0.21-0.33	Cohort	Retrospective (Self-reported)	Kellogg	2005	
Americas	san Francisco	1996-2002	Other	0.82	0.1-2.6	cohort	Retrospective	Kellogg	2005	
Americas	san Francisco	1996	High Risk	1.9		cohort	Retrospective	Kellogg	1999	[262]
Americas	san Francisco	1995	High Risk	2.8		cohort	Retrospective	Kellogg	1999	
Americas	san Francisco	1993-1999	Other	1.4	1.2-1.7	cohort	Retrospective	Kellogg	2001	[260]
Americas	san Francisco	1997-2000	High Risk	7.8	4.6-12.3	cohort	Retrospective	Kellogg	2001	[261]
Americas	Honduras	2007	High Risk	0.23		Other	MOT	Kim	2013	[80]
Americas	Honduras	2007	High Risk	1.03		Other	MOT	Kim	2013	
Americas	Honduras	2007	Other	0.3		Other	MOT	Kim	2013	
Americas	Honduras	2006	High Risk	0	0.-0.01	RITA	BED-CEIA	Kim	2013	
Americas	Honduras	2006	High Risk	1.1	0.2-2.0	RITA	BED-CEIA	Kim	2013	
Americas	Honduras	2006	Other	0.4	0.1-0.8	RITA	BED-CEIA	Kim	2013	
Americas	san Francisco	1999-2001	High Risk	0.4	0.1-2.1	RITA	Detuned	Kim	2008	[288]
Americas	USA	2009-2010	High Risk	3	2.0-4.4	cohort	Prospective	Koblin	2013	[213]
Americas	USA	1999-2001	High Risk	2.1	1.9-2.4	cohort	Prospective	Koblin	2006	[215]
Americas	USA	2009-2010	High Risk	0.31	0.06-0.91	cohort	Prospective	Koblin	2013	[214]
Americas	USA	1987-1998	High Risk	1.2	0.7-2.0	RITA	Detuned	Kral	2003	[289]
Americas	san Francisco	2003	Other	0.56		RITA	Avidity	Laeyendecker	2008	[290]

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Americas	san Francisco	2003	Other	1.9		RITA	Detuned	Laeyendecker	2008	
Americas	san Francisco	2001	Other	0.94		RITA	Avidity	Laeyendecker	2008	
Americas	san Francisco	2001	Other	1.73		RITA	Detuned	Laeyendecker	2008	
Americas	USA	1995-1999	Testing Population	0.97	0.51-1.71	RITA	MAA	Laeyendecker-1	2013	[343]
Americas	Brisitsh Columbia, Canada	2003	High Risk	2.36	0-5.62	cohort	Prospective	Lampinen	2005	[210]
Americas	Brisitsh Columbia, Canada	2002	High Risk	1.53	0-3.25	cohort	Prospective	Lampinen	2005	
Americas	Brisitsh Columbia, Canada	2001	High Risk	0.43	0-1.27	cohort	Prospective	Lampinen	2005	
Americas	Brisitsh Columbia, Canada	2000	High Risk	0.42	0-1.24	cohort	Prospective	Lampinen	2005	
Americas	Brisitsh Columbia, Canada	1999	High Risk	0.86	0-2.04	cohort	Prospective	Lampinen	2005	
Americas	Brisitsh Columbia, Canada	1998	High Risk	0.96	0-2.29	cohort	Prospective	Lampinen	2005	
Americas	Brisitsh Columbia, Canada	1997	High Risk	0.56	0-1.65	cohort	Prospective	Lampinen	2005	
Americas	Montreal	1996-2003	High Risk	0.62	0.41-0.84	cohort	Prospective	Lavoie	2008	[209]
Americas	Denver		Testing Population	0.3	0.1-0.6	RITA	Detuned	Linley	2002	[291]
Americas	Miama		Testing Population	1.2	0.7-2.9	RITA	Detuned	Linley	2002	
Americas	Los Angeles		Testing Population	0.8	0.5-1.3	RITA	Detuned	Linley	2002	
Americas	Newark		Testing Population	0.9	0.5-1.6	RITA	Detuned	Linley	2002	
Americas	USA-Netherlands	1998-1999	High Risk	2.91	2.30-3.53	RITA	BED-CEIA McDougal	McDougal	2006	[202]
Americas	USA-Netherlands	1998-1999	High Risk	3.1	2.57-3.63	cohort	Prospective	McDougal	2006	
Americas	san Francisco	1996-1998	Testing population	1.1	0.68-1.6	RITA	Detuned	McFarland	1999	[292]
Americas	San Francisco	1995-1998	High Risk	0	0-1.90	RITA	Detuned	McFarland	2000	[256]
Americas	San Francisco	1995-1998	High Risk	0	0-1.02	cohort	Retrospective	McFarland	2000	
Americas	san Francisco	1995	High Risk	2.8	2.3-3.4	cohort	Retrospective	McFarland	1997	[257]
Americas	Baltimore	2004	High Risk	0.53	0.16-1.59	cohort	Prospective	Mehta	2006	[198]
Americas	Baltimore	1993-2002	Testing Population	0.91	0.76-1.09	cohort	Retrospective	Mehta	2006	[272]
Americas	Baltimore	1988-2004	High Risk	2.06	1.83-2.31	cohort	Prospective	Mehta	2006	[198]
Americas	Baltimore	1988	High Risk	4.57	3.12-6.71	cohort	Prospective	Mehta	2006	
Americas	USA	1994-2000	High Risk	2.6		RITA	BED-CEIA	Mermin	2008	[328]
Americas	USA	1998-200	High Risk	4.4	2.9-6.7	RITA	Detuned	MMWR	2001	[293]
Americas	san Francisco	1985-1990	High Risk	1.9	1.2-2.8	cohort	Prospective	Moss	1994	[197]
Americas	New York	2001	Testing population	0.29	0.20-0.38	RITA	Detuned	Nash	2005	[295]
Americas	New York	2008	High Risk	5.67		cohort	Retrospective	Neaigus	2012	[255]
Americas	Atlanta	1991-1998	Other	2.4	2.0-2.9	RITA	BED-CEIA	Nesheim	2005	[329]
Americas	North Carolina	2002-2003	Testing population	0.2	0.1-0.3	RITA	Detuned	Pilcher	2005	[297]
Americas	Chicago	2004	Other	0.39	0.36-0.43	RITA	Detuned	Prachand	2006	[298]
Americas	Chicago	2004	High Risk	0.66	0.60-0.73	RITA	Detuned	Prachand	2006	
Americas	Chicago	2004	Testing population	0.96	0.87-1.06	RITA	Detuned	Prachand	2006	
Americas	Chicago	2004	High Risk	3.79	3.44-4.18	RITA	Detuned	Prachand	2006	
Americas	Geogria-USA	2002-2004	High Risk	1.3	0.6-2.1	RITA	BED-CEIA	Priddy	2007	[299]
Americas	Geogria-USA	2002-2004	High Risk	1.1	0.4 to 1.8	RITA	Detuned	Priddy	2007	
Americas	San Francisco	1989-1998	Testing Population	1.0 OR	0.98-1.1	RITA	Detuned	Razani	2006	[300]
Americas	Montreal	1996-2001	High Risk	0.56	0.29-0.83	cohort	Prospective	Remis	2002	[189]
Americas	USA	1985-1999	Other	0.17	0.16-0.17	cohort	Prospective	Renzullo	2001	[187]
Americas	USA	1985-1993	Other	0.027	0.025-0.029	cohort	Prospective	Renzullo	1995	[188]

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Americas	Brazil	2007-2008	Other	0.0385	0.0256-0.0514	RITA	Detuned	Sabino	2012	[301]
Americas	Brazil	2007-2008	Other	0.0226	0.0171-0.0280	RITA	Detuned	Sabino	2012	
Americas	Brazil	1998	High Risk	2.8	1.4-5.3	RITA	Detuned	Schechter	2000	[302]
Americas	Brazil	1998	High Risk	1.9	0.9-3.9	RITA	Detuned	Schechter	2000	
Americas	Brazil	1995-1998	High Risk	2.9	1.4-5.1	cohort	Prospective	Schechter	2004	[181]
Americas	san Francisco	2009	Other	0.052	0.037-0.067	RITA	BED-CEIA	Scheer	2012	[334]
Americas	California (Excluding Los Angeles and San Francisco)	2009	Other	0.015	0.011-0.020	RITA	BED-CEIA	Scheer	2012	
Americas	Los Angeles	2009	Other	0.023	0.017-0.028	RITA	BED-CEIA	Scheer	2012	
Americas	san Francisco	2008	Other	0.065	0.049-0.082	RITA	BED-CEIA	Scheer	2012	
Americas	California (Excluding Los Angeles and San Francisco)	2008	Other	0.013	0.009-0.017	RITA	BED-CEIA	Scheer	2012	
Americas	Los Angeles	2008	Other	0.020	0.016-0.024	RITA	BED-CEIA	Scheer	2012	
Americas	san Francisco	2007	Other	0.070	0.048-0.092	RITA	BED-CEIA	Scheer	2012	
Americas	Los Angeles	2007	Other	0.029	0.022-0.036	RITA	BED-CEIA	Scheer	2012	
Americas	san Francisco	2006	Other	0.070	0.047-0.094	RITA	BED-CEIA	Scheer	2012	
Americas	San Francisco	1998-1999	Testing Population	1.5	0.8-2.8	RITA	Detuned	Schwarcz	2002	[304]
Americas	San Francisco	1989-1998	Testing Population	1.6		RITA	Detuned	Schwarcz	2001	
Americas	USA	1995-1997	High Risk	0.38	0.14-1.01	cohort	Prospective	Seage	2001	[180]
Americas	USA	1995-1997	High Risk	1.24	0.56-2.77	cohort	Prospective	Seage	2001	
Americas	USA	1995-1997	High Risk	1.55	1.23-1.95	cohort	Prospective	Seage	2001	
Americas	USA	1995-1997	High Risk	1.13	0.57-2.27	cohort	Prospective	Seage	2001	
Americas	USA	1995-1997	High Risk	1.31	1.06-1.61	cohort	Prospective	Seage	2001	
Americas	Vancouver	1996-2000	High Risk	13.4	11.0-15.8	cohort	Prospective	Spittal	2002	[174]
Americas	Baltimore	1988-1998	High Risk	3.14	2.78-3.53	cohort	Prospective	Strathdee	2001	[173]
Americas	Seattle	1995	High Risk	1.3		cohort	Prospective	Tabet	1998	[172]
Americas	Los Angeles	2002-2004	High Risk	17	12-22	RITA	Detuned	Taylor	2005	[305]
Americas	King County, Washington	2001-2004	Testing population	1.3	0.8-2.0	RITA	Detuned	Thibault	2007	[306]
Americas	King County, Washington	2001-2004	Testing population	0.4	0.3-6.0	RITA	Detuned	Thibault	2007	
Americas	USA	2004	High Risk	4.01		RITA	BED-CEIA McDougal	Troung	2009	[252]
Americas	USA	2004	High Risk	4.06		RITA	Detuned Mcdougal	Troung	2009	
Americas	USA	2004	High Risk	4.11		cohort	Retrospective	Troung	2009	
Americas	USA	2003	High Risk	2.26		RITA	BED-CEIA McDougal	Troung	2009	
Americas	USA	2003	High Risk	3.74		RITA	BED-CEIA McDougal	Troung	2009	
Americas	USA	2003	High Risk	1.78		cohort	Retrospective	Troung	2009	
Americas	USA	2003	High Risk	4.09		RITA	Detuned Mcdougal	Troung	2009	
Americas	USA	2003	High Risk	2.07		RITA	Detuned Mcdougal	Troung	2009	
Americas	USA	2003	High Risk	2.78		cohort	Retrospective	Troung	2009	
Americas	USA	2002	High Risk	1.93		cohort	Retrospective	Troung	2009	
Americas	USA	2002	High Risk	2.91		RITA	Detuned Mcdougal	Troung	2009	
Americas	USA	2002	High Risk	4.46		RITA	BED-CEIA McDougal	Troung	2009	
Americas	USA	2002	High Risk	3.83		RITA	BED-CEIA McDougal	Troung	2009	
Americas	USA	2002	High Risk	4.17		RITA	Detuned Mcdougal	Troung	2009	
Americas	USA	2002	High Risk	2.97		cohort	Retrospective	Troung	2009	
Americas	USA	2001	High Risk	3.46		RITA	Detuned Mcdougal	Troung	2009	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Americas	USA	2001	High Risk	3.01		cohort	Retrospective	Troung	2009	
Americas	USA	2001	High Risk	2.36		RITA	BED-CEIA McDougal	Troung	2009	
Americas	USA	2001	High Risk	2.71		RITA	Detuned Mcdougal	Troung	2009	
Americas	USA	2001	High Risk	2.57		cohort	Retrospective	Troung	2009	
Americas	USA	2001	High Risk	4.06		RITA	BED-CEIA McDougal	Troung	2009	
Americas	USA	2000	High Risk	3.36		RITA	BED-CEIA McDougal	Troung	2009	
Americas	USA	2000	High Risk	3.66		RITA	Detuned Mcdougal	Troung	2009	
Americas	USA	2000	High Risk	4		RITA	BED-CEIA McDougal	Troung	2009	
Americas	USA	2000	High Risk	3.28		cohort	Retrospective	Troung	2009	
Americas	USA	2000	High Risk	3.52		RITA	Detuned Mcdougal	Troung	2009	
Americas	USA	2000	High Risk	1.9		cohort	Retrospective	Troung	2009	
Americas	san Francisco	2004	High Risk	3.2		RITA	Detuned	Truong	2006	[308]
Americas	san Francisco	2004	High Risk	3.6		RITA	Detuned	Truong	2006	
Americas	san Francisco	2003	High Risk	1.7		RITA	Detuned	Truong	2006	
Americas	san Francisco	2003	High Risk	4.2		RITA	Detuned	Truong	2006	
Americas	san Francisco	2002	High Risk	2.8		RITA	Detuned	Truong	2006	
Americas	san Francisco	2002	High Risk	4.4		RITA	Detuned	Truong	2006	
Americas	san Francisco	2001	High Risk	2.8		RITA	Detuned	Truong	2006	
Americas	san Francisco	2001	High Risk	3.4		RITA	Detuned	Truong	2006	
Americas	san Francisco	2000	High Risk	2.9		RITA	Detuned	Truong	2006	
Americas	san Francisco	2000	High Risk	3.3		RITA	Detuned	Truong	2006	
Americas	san Francisco	1999	High Risk	4.8		RITA	Detuned	Truong	2006	
Americas	san Francisco	1999	High Risk	4.1		RITA	Detuned	Truong	2006	
Americas	san Francisco	1998	High Risk	4.6		RITA	Detuned	Truong	2006	
Americas	san Francisco	1998	High Risk	1.1		RITA	Detuned	Truong	2006	
Americas	Brazil		Other	1.68	1.29-2.10	RITA	BED-CEIA	Velasco de Castro	2010	[339]
Americas	Brazil		Other	1.09	1.32-1.87	RITA	BED-CEIA McDougal	Velasco de Castro	2010	
Americas	Brazil		Other	1.17	0.88-1.47	RITA	BED-CEIA Hargrove	Velasco de Castro	2010	
Americas	Argentina	2000	Other	10.19		RITA	Detuned	Vignoles	2002	[310]
Americas	Argentina	1999	Other	4.62		RITA	Detuned	Vignoles	2002	
Americas	Argentina	1998	Other	0.4		RITA	Detuned	Vignoles	2002	
Americas	USA	2006-2008	Other	0.5	0.3-1.0	cohort	Prospective	Wallrauch	2010	[169]
Americas	New York	1994-1996	Testing Population	0.79	0.52-1.2	cohort	Retrospective	Weinstock	1998	[251]
Americas	Los Angeles	1994-1996	Testing Population	0.29	0.12-0.69	cohort	Retrospective	Weinstock	1998	
Americas	Houston	1993-1996	Testing Population	1.21	0.73-2.0	cohort	Retrospective	Weinstock	1998	
Americas	Baltimore	1992-1996	Testing Population	0.81	0.67-0.97	cohort	Retrospective	Weinstock	1998	
Americas	USA	1991-1997	Testing Population	0.8	0.6-1.1	RITA	Detuned	Weinstock	2002	[311]
Americas	New Orleans	1991-1996	Testing Population	0.57	0.47-0.70	cohort	Retrospective	Weinstock	1998	[251]
Americas	Atlanta	1991-1996	Testing Population	0.33	0.22-0.48	cohort	Retrospective	Weinstock	1998	
Americas	Denver	1991-1996	Testing Population	0.089	0.048-0.17	cohort	Retrospective	Weinstock	1998	
Americas	USA	1991-1996	Testing Population	0.52	0.46-0.58	cohort	Retrospective	Weinstock	1998	
Americas	Seattle	1996-2000	High Risk	1.76	1.04-2.75	RITA	Detuned Satten	White	2010	[162]
Americas	Seattle	1996-2000	High Risk	1.14	0.89-1.43	cohort	Prospective	White	2010	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Americas	Seattle	1996-2000	High Risk	1.7	0.98-2.51	RITA	Detuned	White	2010	
Americas	Vancouver	1996-2007	High Risk	2.49	2.09-2.88	cohort	Prospective	Wood	2009	[161]
Americas	Vancouver	1996-2002	High Risk	3.2	2.6-3.8	cohort	Prospective	Wood	2005	
Americas	Rio De Janeiro, Brazil	1999-2001	High Risk	2.86	1.04-4.68	RITA	BED-CEIA	Teixeira	2004	[312]
Americas	Miami, Florida	1991	High Risk	18		Cohort		Onorato	1995	[462]
Americas	Miami, Florida	1990	High Risk	19		Cohort		Onorato	1995	
Americas	Miami, Florida	1989	High Risk	16		Cohort		Onorato	1995	
Americas	Miami, Florida	1988	High Risk	12		Cohort		Onorato	1995	
Americas	Buenos Aires	2003-2004	High Risk	3.9	2.0-6.7	Cohort	Prospective	Segura	2007	[153]
Americas	California	2006	Testing Population	1.9	1.7-2.0	Cohort	Retrospective	Xia	2011	[246]
Americas	California	2003	Testing Population	2.4	2.2-2.6	Cohort	Retrospective	Xia	2011	
Americas	California	1997	Testing Population	2.0	1.8-2.2	Cohort	Retrospective	Xia	2011	
Americas	Baltimore	1995-1998	High Risk	1.84		Cohort	Prospective	Nelson	2002	[148]
Americas	Baltimore	1991-1994	High Risk	3.35		Cohort	Prospective	Nelson	2002	
Americas	Baltimore	1988-1990	High Risk	4.45		Cohort	Prospective	Nelson	2002	
Americas	Montreal	1992-2008	High Risk	3.3	2.8-3.9	Cohort	Prospective	Bruneau	2010	[143]
Americas	Canada	1995-2009	High Risk	2.7	2.4-2.1	Cohort	Prospective	Roy	2011	[152]
Americas	Chicargo	1994-1996	High Risk	1.1		Cohort	Prospective	Ouellet	2000	[149]
Asia - Australiasia	Thailand		High Risk	17.3	2.1-32.5	RITA	BED-CEIA	Apornpong	2007	[314]
Asia - Australiasia	Thailand		Testing population	5.8	2.02-9.7	RITA	BED-CEIA	Apornpong	2007	
Asia - Australiasia	Thailand		High Risk	25.4	14.8-40.6	RITA	p24 antigen - FSW	Beyrer	1996	[241]
Asia - Australiasia	Thailand		High Risk	19.9	4.1-58.1	RITA	p24 antigen - MSW	Beyrer	1996	
Asia - Australiasia	Thailand		High Risk	25.4	13.3-39.3	Cohort	Cohort - FSW	Beyrer	1996	
Asia - Australiasia	Thailand		High Risk	11.9	7.8-17.4	Cohort	Cohort - MSW	Beyrer	1996	
Asia - Australiasia	Pune-India	1993-1994	Testing Population	11.7	8.1-16.5	Cohort	Cohort	Brookmeyer	1995	[114]
Asia - Australiasia	Pune-India	1993-1994	Testing Population	19.6	11-32.3	RITA	p24 antigen	Brookmeyer	1995	
Asia - Australiasia	Thailand	1993-1995	High Risk	18.6	14.4-23.9	cohort	Retrospective	Celentano	1999	[267]
Asia - Australiasia	India	2004-2005	Other	0.125	0.082-0.168	cohort	Prospective	Dandona	2013	[235]
Asia - Australiasia	Dehong Prefecture-China	2008	High Risk	4.3		RITA	BED-CEIA Hargrove	Duan	2010	[318]
Asia - Australiasia	Dehong Prefecture-China	2008	High Risk	4.7		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2008	Other	0.13		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2008	High Risk	1.3		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2008	Other	0.1		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2007	Other	0.1		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2007	High Risk	9.7		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2007	High Risk	7.2		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2007	High Risk	0.6		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2007	Other	0.16		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2006	High Risk	11.5		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2006	Other	0.1		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2006	Other	0.16		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2006	High Risk	2.3		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2006	High Risk	3.4		RITA	BED-CEIA Hargrove	Duan	2010	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Asia - Australiasia	Dehong Prefecture-China	2005	High Risk	9.9		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2005	High Risk	4		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2005	Other	0.1		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2005	High Risk	0.9		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2004	Other	0.1		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2004	High Risk	15		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2004	High Risk	1.4		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Dehong Prefecture-China	2004	High Risk	5.5		RITA	BED-CEIA Hargrove	Duan	2010	
Asia - Australiasia	Calcutta, India	2005	High Risk	8.3	4.3-12.2	RITA	Detuned	Gupta	2003	[283]
Asia - Australiasia	Calcutta, India	2004	High Risk	6.9	3.5-10.4	RITA	Detuned	Gupta	2003	
Asia - Australiasia	Bangkok	1996	High Risk	9	6.7-11.9	cohort	Prospective	Hu	2003	[224]
Asia - Australiasia	Bangkok	1996	High Risk	17.3	12.8-24.2	RITA	BED-CEIA	Hu	2003	
Asia - Australiasia	China	2006	High Risk	0.9	0-2.67	RITA	BED-CEIA	Jiang	2007	[321]
Asia - Australiasia	China	2006	High Risk	1.06	0-2.25	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2005	High Risk	2.07	1.26-2.89	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2005	High Risk	9.58	5.83-13.34	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2005	High Risk	1.04	0-3.07	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2005	High Risk	1.12	0.46-1.79	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2004	High Risk	0.94	0-2.78	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2004	High Risk	1.05	0.36-1.73	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2003	High Risk	7.89	5.07-10.71	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2002	High Risk	8.17	5.19-11.14	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2002	High Risk	0.93	0.11-1.74	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2001	High Risk	9.77	6.91-12.62	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2001	High Risk	0.57	0-1.22	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	China	2000	High Risk	9.15	6.42-11.89	RITA	BED-CEIA	Jiang	2007	
Asia - Australiasia	Thailand	2006 (November)	Other	0.18	0.10-0.25	RITA	BED-CEIA	Kana	2008	[322]
Asia - Australiasia	Thailand	2006 (May)	Other	0.22	0.14-0.36	RITA	BED-CEIA	Kana	2008	
Asia - Australiasia	Thailand	2005	Other	0.15	0.09-0.21	RITA	BED-CEIA	Kana	2008	
Asia - Australiasia	Thailand	1991-1994	High Risk	4.3	2.9-6.2	cohort	Prospective	Kilmarx	1998	[217]
Asia - Australiasia	Thailand	1987-1992	High Risk	18.2		cohort	Prospective	Kitayaporn	1994	[216]
Asia - Australiasia	India		Other	0.32	0.20-0.44	RITA	BED-CEIA	Lakshmi	2009	[325]
Asia - Australiasia	Beijing-China	2009	High Risk	8.09	6.92-9.26	cohort	Prospective	Li	2012	[207]
Asia - Australiasia	Beijing-China	2006-2007	High Risk	2.6	1.1-4.1	cohort	Prospective	Li	2010	[208]
Asia - Australiasia	Beijing-China	2006-2007	High Risk	2.6	1.1-4.1	cohort	Prospective	Li	2010	
Asia - Australiasia	Beijing-China	2006	High Risk	3.6	1.3-5.9	RITA	BED-CEIA	Li	2008	[327]
Asia - Australiasia	Bejing	2005	High Risk	3.19	0.8-5.6	RITA	BED-CEIA	Li	2007	[326]
Asia - Australiasia	Beijing-China	2005	High Risk	2.9	0.8-5.0	RITA	BED-CEIA	Li	2008	[327]
Asia - Australiasia	Thailand	2000-2002	Testing Population	3.75	2.77-4.73	RITA	BED-CEIA Welte	McNicholl	2011	[201]
Asia - Australiasia	Thailand	2000-2002	Testing Population	3.73	2.78-4.68	RITA	BED-CEIA without LT	McNicholl	2011	
Asia - Australiasia	Thailand	2000-2002	Testing Population	4.41	3.43-5.39	RITA	BED-CEIA with LT	McNicholl	2011	
Asia - Australiasia	Thailand	2000-2002	Testing Population	3.71	3.19-4.22	cohort	Prospective	McNicholl	2011	
Asia - Australiasia	Thailand	1995-1998	High Risk	5.49	2.22-8.76	RITA	BED-CEIA	McNicholl	2011	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Asia - Australiasia	Thailand	1995-1998	High Risk	6.96	5.67-8.25	cohort	Prospective	McNicholl	2011	
Asia - Australiasia	Thailand	1991-1993	Other	1.43	0.36-2.49	RITA	BED-CEIA	McNicholl	2011	
Asia - Australiasia	Thailand	1991-1993	Other	1.19	0.56-1.82	cohort	Prospective	McNicholl	2011	
Asia - Australiasia	"Pure, India"	2002-2004	High Risk	1.22	0.45-2.66	cohort	Prospective	Mehendale	2006	[200]
Asia - Australiasia	India	1993-1995	Testing Population	10.2	7.9-13.1	cohort	Prospective	Mehendale	1995	[199]
Asia - Australiasia	China	2008	High Risk	5.6		cohort	Prospective	Peng	2012	[194]
Asia - Australiasia	Thailand	2005	High Risk	1.2		RITA	BED-CEIA	Plipat	2006	[330]
Asia - Australiasia	Thailand	2005	Other	0.2		RITA	BED-CEIA	Plipat	2006	
Asia - Australiasia	Thailand	2005	High Risk	0.9		RITA	BED-CEIA	Plipat	2006	
Asia - Australiasia	Thailand	2004	High Risk	3.7		RITA	BED-CEIA	Plipat	2006	
Asia - Australiasia	Thailand	2004	High Risk	0.8		RITA	BED-CEIA	Plipat	2006	
Asia - Australiasia	Thailand	2004	Other	0.2		RITA	BED-CEIA	Plipat	2006	
Asia - Australiasia	Australia	2001-2004	Other	0.78	0.59-1.02	cohort	Prospective	Poynten	2010	[193]
Asia - Australiasia	Pune, India	1993-1994	Testing Population	18.6	8.9-34.2	RITA	HIV RNA	Quinn	2000	[190]
Asia - Australiasia	Pune, India	1993-1994	Testing Population	9.4	4.8-16.4	Cohort	Cohort	Quinn	2000	
Asia - Australiasia	Pune, India	1993-1994	Testing Population	18.5	8-36.5	RITA	p24 antigen	Quinn	2000	
Asia - Australiasia	Beijing-China	2006-2007	High Risk	2.6	1.1-4.1	cohort	Prospective	Ruan	2009	[185]
Asia - Australiasia	Beijing-China	2006-2007	High Risk	2.6	1.1-4.1	cohort	Prospective	Ruan	2009	
Asia - Australiasia	Sichuan Province, China	2002	High Risk	3.17	0.98-5.37	cohort	Prospective	Ruan	2005	[184]
Asia - Australiasia	Cambodia	2006	High Risk	3.3	1.7-4.9	RITA	BED-CEIA	Saphonn	2007	[333]
Asia - Australiasia	Cambodia	2002	Other	0.59		RITA	BED-CEIA	Saphonn	2005	[332]
Asia - Australiasia	Cambodia	2002	High Risk	2.87		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	2002	High Risk	6.45		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	2002	Other	0.26		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	2000	Other	1.30		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	2000	Other	1.11		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	2000	High Risk	9.02		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	2000	High Risk	5.08		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	1999	High Risk	5.08		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	1999	Other	0.72		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	1999	Other	1.74		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Cambodia	1999	High Risk	13.90		RITA	BED-CEIA	Saphonn	2005	
Asia - Australiasia	Ho Chi Minh, Vietnam		High Risk	2.1	0.8-3.4	RITA	Avidity	Sexton	2012	[178]
Asia - Australiasia	Ho Chi Minh, Vietnam		High Risk	0	0-4.1	cohort	Prospective	Sexton	2012	
Asia - Australiasia	Ho Chi Minh, Vietnam		High Risk	0.8	0.1-1.5	RITA	BED-CEIA	Sexton	2012	
Asia - Australiasia	Ho Chi Minh, Vietnam		High Risk	0.5	0-1.0	RITA	BED-CEIA Hargrove	Sexton	2012	
Asia - Australiasia	Ho Chi Minh, Vietnam		High Risk	0.5	0-1.3	RITA	BED-CEIA Welte	Sexton	2012	
Asia - Australiasia	Ho Chi Minh, Vietnam		High Risk	1.7	0.6-2.8	RITA	Avidity Hargrove	Sexton	2012	
Asia - Australiasia	Ho Chi Minh, Vietnam		High Risk	1.7	0.4-3.0	RITA	Avidity Welte	Sexton	2012	
Asia - Australiasia	Thailand	1991 (5mth)	Other	0.55	-0.2-1.3	RITA	BED-CEIA	Sinthuwattaniwibool	2005	[175]
Asia - Australiasia	Thailand	1991 (5mth)	Other	0.62	0.1-1.1	cohort	Prospective	Sinthuwattaniwibool	2005	
Asia - Australiasia	Thailand	1991 (23mth)	Other	0.51	-0.5-1.5	RITA	BED-CEIA	Sinthuwattaniwibool	2005	
Asia - Australiasia	Thailand	1991 (23mth)	Other	1.45	0.4-2.5	cohort	Prospective	Sinthuwattaniwibool	2005	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Asia - Australiasia	Thailand	1991 (17mth)	Other	1.44	0.03-2.9	RITA	BED-CEIA	Sinthuwattaniwibool	2005	
Asia - Australiasia	Thailand	1991 (17mth)	Other	1.36	0.5-2.3	cohort	Prospective	Sinthuwattaniwibool	2005	
Asia - Australiasia	India	2004	High Risk	4.66	2.02-7.30	RITA	BED-CEIA	Srikrishnan	2006	[335]
Asia - Australiasia	Thailand	2009	Other	0.25	0.17-0.32	RITA	BED-CEIA	Tabprasit	2007	[336]
Asia - Australiasia	Thailand	2006-2008	Other	0.26	0.19-0.36	RITA	BED-CEIA	Tabprasit	2007	
Asia - Australiasia	Thailand	2005	Other	0.14	0.09-0.20	RITA	BED-CEIA	Tabprasit	2007	
Asia - Australiasia	Mumbai-India	2002-2004	High Risk	6.81	5.42-8.21	RITA	BED-CEIA Adjusted	Troung	2008	[337]
Asia - Australiasia	Cambodia		Other	1.33		RITA	Detuned	Troung	2004	[307]
Asia - Australiasia	Cambodia		Other	4.35		RITA	BED-CEIA	Troung	2004	
Asia - Australiasia	Cambodia		Other	3.52		RITA	Detuned	Troung	2004	
Asia - Australiasia	Thailand	2006-2012	High Risk	5.9		cohort	Prospective	Van Griensven	2013	[171]
Asia - Australiasia	Bangkok	1995-1996	High Risk	5.8	4.8-6.8	cohort	Prospective	Vanichseni	2001	[170]
Asia - Australiasia	China	2006-2009	High Risk	1.44	0.87-2.24	cohort	Prospective	Wang	2012	[165]
Asia - Australiasia	Kaiyuan City-China	2006	High Risk	2.6	0.3-9.2	Cohort	Prospective (Close Chort)	Wang	2007	[164]
Asia - Australiasia	Kaiyuan City-China	2006	High Risk	2.1	0.4-3.8	RITA	BED-CEIA	Wang	2007	
Asia - Australiasia	Kaiyuan City-China	2006	High Risk	1.4	0.2-4.9	Cohort	Prospective (Open Chort)	Wang	2007	
Asia - Australiasia	Kaiyuan City-China		High Risk	0.8	0.2-1.4	RITA	BED-CEIA	Wang	2008	[340]
Asia - Australiasia	Bangkok	1999-2000	High Risk	7.4		RITA	BED-CEIA	Wasinrapee	2004	[341]
Asia - Australiasia	Guangxi, China	2002	High Risk	3.1	1.6-5.2	cohort	Prospective	Wei	2006	[163]
Asia - Australiasia	Kaiyuan City-China	2006-2007	High Risk	1.1	0.3-2.8	cohort	Prospective	Xu	2010	[157]
Asia - Australiasia	Kaiyuan City-China	2006-2007	High Risk	3.4	2.3-4.4	RITA	BED-CEIA	Xu	2010	
Asia - Australiasia	Kaiyuan City-China	2006-2007	High Risk	1.5	1.0-2.0	RITA	BED-CEIA McDougal	Xu	2010	
Asia - Australiasia	Kaiyuan City-China	2006-2007	High Risk	1.6	1.1-2.1	RITA	BED-CEIA Hargrove	Xu	2010	
Asia - Australiasia	Yunnan, China	2006-2007	High Risk	4.4	2.8-6.0	RITA	BED-CEIA	Xu	2013	[353]
Asia - Australiasia	Yunnan, China	2006-2007	High Risk	2.9	1.9-4.0	RITA	BED-CEIA McDougal	Xu	2013	
Asia - Australiasia	Yunnan, China	2006-2007	High Risk	3.2	2.0-4.3	RITA	BED-CEIA Hargrove	Xu	2013	
Asia - Australiasia	China	2009-2011	High Risk	3.5	1.8-6.2	cohort	Prospective	Xu	2013	[159]
Asia - Australiasia	China	2006	High Risk	5.4	2.0-11.3	cohort	Prospective	Xu	2010	[158]
Asia - Australiasia	China	2008-2010	High Risk	3.36	2.13-5.04	cohort	Prospective	Yan	2012	[56]
Asia - Australiasia	Naujing-China	2008	High Risk	5.12	1.33-8.91	cohort	Prospective	Yang	2010	[156]
Asia - Australiasia	Xinjiang, China	2002	High Risk	8.8	6.3-12.0	cohort	Prospective	Zhang	2007	[155]
Asia - Australiasia	Pure, India	1993-2002	Testing Population	3.94	3.45-4.47	Cohort	Prospective	Mehendale	2007	[147]
Asia - Australiasia	Thailand	1989-1997	High Risk	5.11	3.13-8.35	Cohort	Retrospective	Sawanpaujalert	1999	[247]
Asia - Australiasia	Australia	1993-1999	Testing Population	2.1		Cohort	Retrospective	McDonald	2001	[248]
Asia - Australiasia	Chennai, India	2005-2006	High Risk	0.48	0.17-1.03	Cohort	Prospective	Solomon	2010	[154]
Asia - Australiasia	Chennai, India	2005-2006	High Risk	2.95	1.21-4.69	RITA	BED-CEIA	Solomon	2010	
Asia - Australiasia	Bangkok, Thailand	1990-1993	Testing Population	0.307		Cohort	Retrospective	Kitayaporn	1996	[249]
Asia - Australiasia	Thailand	1993-1995	Other	0.55		Cohort	Prospective	Celentano	1998	[144]
Asia - Australiasia	Thailand	1991-1993	Other	2.48		Cohort	Prospective	Celentano	1998	
Asia - Australiasia	Thailand	1993-1997	High Risk	11.44		Cohort	Retrospective	Jittiwatikarn	2000	[250]
Eastern Europe	Russia	2005-2006	High Risk	23.9		RITA	BED-CEIA McDougal	Heimer	2010	[263]
Eastern Europe	Russia	2005-2006	High Risk	25.5		RITA	BED-CEIA Hargrove	Heimer	2010	
Eastern Europe	Russia	2005-2006	High Risk	3.8		RITA	BED-CEIA Hargrove	Heimer	2010	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Eastern Europe	Russia	2005-2006	High Risk	3.6		RITA	BED-CEIA McDougal	Heimer	2010	
Eastern Europe	Russia		High Risk	14.1-1.9		cohort	Retrospective	Heimer	2010	
Eastern Europe	Russia	2002	High Risk	4.5	2.7-7.0	cohort	Prospective	Kozlov	2006	[212]
Eastern Europe	Russia	2005-2008	High Risk	23.9	17.8-30.1	RITA	BED-CEIA Hargrove	Niccolai	2010	[254]
Eastern Europe	Russia	2005-2008	High Risk	25.5	18.9-32.0	RITA	BED-CEIA McDougal	Niccolai	2010	
Eastern Europe	Russia	2005-2008	High Risk	14.1	10.7-17.6	cohort	Retrospective	Niccolai	2010	
Eastern Europe	Russia		High Risk	6.5	3.1-9.2	RITA	Detuned	Verevochkin	2008	[309]
Eastern Europe	Russia		High Risk	12.7		RITA	BED-CEIA	Verevochkin	2008	
Eastern Europe	Estonia	2005	High Risk	21		Other	Cross-sectional	Uuskula	2008	[463]
Eastern Europe	Estonia	2004	High Risk	31		Other	Cross-sectional	Uuskula	2008	
Western Europe	Portugal	2005	Testing population	4.1		RITA	Avidity	Cortes Martins	2008	[347]
Western Europe	Portugal	2004	Testing Population	3.3		RITA	Avidity	Cortes Martins	2008	
Western Europe	Spain		High Risk	1.06-4.71	NR	cohort	Prospective	Del Romero	2001	[234]
Western Europe	Amsterdam	1999-2005	Testing Population	3.75	2.37-5.77	RITA	Detuned	Dukers	2007	[232]
Western Europe	Amsterdam	1999-2005	High Risk	1.24	0.89-1.72	cohort	Prospective	Dukers	2007	
Western Europe	Rotterdam	1999-2003	High Risk	1.53	0.82-2.84	cohort	Prospective	Dukers	2007	
Western Europe	Amsterdam	1991-2001	High Risk	3.0	1.8-4.6	RITA	Detuned	Dukers	2002	[281]
Western Europe	Amsterdam	1991-1998	High Risk	1.13	0.82-1.54	cohort	Prospective	Dukers	2007	[232]
Western Europe	Amsterdam	1991-1998	Testing Population	1.81	0.94-3.38	RITA	Detuned	Dukers	2007	
Western Europe	Amsterdam	1984-1990	High Risk	3.34	2.75-1.54	cohort	Prospective	Dukers	2007	
Western Europe	UK	1997-1998	High Risk	1.8	0.9-3.2	cohort	Retrospective	Elford	2001	[265]
Western Europe	Italy	2000-2003	High Risk	4.97	3.52-7.03	cohort	Retrospective	Giuliani	2005	[264]
Western Europe	Italy	1996-1999	High Risk	2.86	1.98-4.01	cohort	Retrospective	Giuliani	2005	
Western Europe	Italy	1984-1995	High Risk	2.26	1.76-2.90	cohort	Retrospective	Giuliani	2005	
Western Europe	Amsterdam	2000-2009	High Risk	6.4	3.4-11.2	cohort	Prospective	Heuker	2012	[227]
Western Europe	Amsterdam	2000-2009	High Risk	1.6	1.3-2.1	cohort	Prospective	Heuker	2012	
Western Europe	Spain	2003	High Risk	3.28	1.2-8.7	cohort	Prospective	Hurtado	2006	[223]
Western Europe	Spain	2003	Testing population	1.15	0.4-3.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2002	Other	0.59	0.1-2.3	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2002	High Risk	1.91	0.8-4.6	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2002	Testing population	1.48	0.8-2.7	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2002	High Risk	4.39	1.6-11.7	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2001	Other	0.29	0.04-2.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2001	High Risk	1.51	0.6-3.6	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2001	High Risk	5.53	2.8-11.0	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2001	Testing population	1.6	0.9-2.7	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2000	Testing population	1.54	0.9-2.5	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2000	Other	0.57	0.1-2.2	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2000	High Risk	3.86	1.9-7.7	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	2000	High Risk	1.41	0.6-3.4	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1999	High Risk	1.58	0.7-3.5	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1999	High Risk	5.52	3.3-9.2	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1999	Testing population	2.06	1.3-3.1	cohort	Prospective	Hurtado	2006	

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Western Europe	Spain	1999	Other	0.28	0.03-2.0	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1998	High Risk	0.49	0.1-2.0	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1998	High Risk	2.51	1.3-4.8	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1998	Testing population	0.9	0.5-1.6	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1997	Other	0.71	0.2-2.2	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1997	High Risk	0.45	0.1-1.8	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1997	High Risk	4.26	2.7-6.7	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1997	Testing population	1.7	1.1-2.6	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1996	Other	0.69	0.2-2.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1996	High Risk	1.36	0.6-3.0	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1996	Testing population	1.91	1.3-2.8	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1996	High Risk	4.08	2.5-6.6	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1995	Other	1.69	0.8-3.5	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1995	Testing population	1.46	0.9-2.3	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1995	High Risk	1.64	0.8-3.4	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1995	High Risk	1.22	0.5-2.9	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1994	High Risk	5.71	3.8-8.6	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1994	Testing population	2.91	2.1-4.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1994	Other	0.55	0.1-2.2	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1994	High Risk	2.58	1.4-4.8	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1993	High Risk	0.96	0.3-3.0	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1993	High Risk	7.34	5.1-10.5	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1993	Other	0.7	0.2-2.8	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1993	Testing population	3.31	2.4-4.6	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1992	High Risk	1.43	0.4-4.4	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1992	Testing population	3.85	2.7-5.4	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1992	High Risk	7.7	5.3-11.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1991	Other	2.61	1.0-6.9	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1991	High Risk	1.55	0.4-6.2	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1991	High Risk	8.84	6.1-12.7	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1991	Testing population	5.52	4.0-7.7	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1990	Testing population	6.37	4.4-9.2	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1990	High Risk	10.07	6.7-15.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1990	High Risk	5.56	2.3-13.4	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1989	High Risk	5.33	1.7-16.5	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1989	High Risk	12.3	7.8-19.3	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1989	Testing population	8.06	5.3-12.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1989	Other	1.56	0.2-11.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1988	High Risk	6.22	2.3-16.6	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1988	Testing population	7.06	3.5-14.1	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1988	High Risk	8.34	2.1-33.3	cohort	Prospective	Hurtado	2006	
Western Europe	Spain	1988	Other	9.59	2.4-38.3	cohort	Prospective	Hurtado	2006	
Western Europe	Amsterdam	2009	High Risk	2.0	1.00-3.75	cohort	Prospective	Jansen	2011	[222]

Region	Region-Country	Time Period	Population Group	Incidence rate	95% CI	MedthodSimple	Method	First Author	Year	Reference
Western Europe	Amsterdam	1996	High Risk	1.4	0.64-2.63	cohort	Prospective	Jansen	2011	
Western Europe	Amsterdam	1993	High Risk	0.2	0.01-1.12	cohort	Prospective	Jansen	2011	
Western Europe	Amsterdam	1992	High Risk	1.3	0.52-2.70	cohort	Prospective	Jansen	2011	
Western Europe	Amsterdam	1985	High Risk	8.6	6.18-11.79	cohort	Prospective	Jansen	2011	
Western Europe	Paris-france	2009	High Risk	3.8	1.5-6.2	RITA	EIA-RI	Le Vu	2012	[350]
Western Europe	Italy	2008	High Risk	0.577		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	[348]
Western Europe	Italy	2008	Testing population	0.0199		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2008	High Risk	0.691		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2007	High Risk	0.043		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2007	Testing population	0.0208		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2007	High Risk	0.601		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2006	High Risk	0.893		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2006	High Risk	1.247		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2006	Testing population	0.0268		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2005	High Risk	1.659		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2005	High Risk	0.864		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2005	Testing population	0.0262		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2004	High Risk	0.834		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2004	Testing population	0.0255		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	Italy	2004	High Risk	1.368		RITA	Avidity-previous Negative-RNA/p24	Mammone	2012	
Western Europe	UK - Scotland	1988-2009	Other	0.21	0.18-0.24	cohort	Retrospective	McDonald	2013	[258]
Western Europe	UK - Scotland	1988-2009	High Risk	0.27	0.22-0.32	cohort	Retrospective	McDonald	2013	
Western Europe	UK - Scotland	1988-2009	High Risk	1.52	1.38-1.70	cohort	Retrospective	McDonald	2013	
Western Europe	UK - Scotland	1988-2009	Testing population	0.37	0.34-0.40	cohort	Retrospective	McDonald	2013	
Western Europe	UK	2001	High Risk	2.45	1.66-3.50	RITA	Detuned	Murphy	2004	[294]
Western Europe	UK	2000	High Risk	2.41	1.55-3.58	RITA	Detuned	Murphy	2004	
Western Europe	UK	1999	High Risk	1.46	0.77-2.66	RITA	Detuned	Murphy	2004	
Western Europe	UK	1998	High Risk	2.33	1.39-3.86	RITA	Detuned	Murphy	2004	
Western Europe	UK	1997	High Risk	2.43	1.45-4.02	RITA	Detuned	Murphy	2004	
Western Europe	UK	1996	High Risk	3.29	2.06-5.23	RITA	Detuned	Murphy	2004	
Western Europe	UK	1995	High Risk	3	1.87-4.82	RITA	Detuned	Murphy	2004	
Western Europe	Italy	1991-1996	Testing Population	1.7	1.2-2.2	cohort	Retrospective	Suligoi	1999	[253]
Western Europe	Valenica, Spain	1988-1992	High Risk	12.02	9.62-14.41	Cohort	Prospective	Rebagliato	1995	[151]
Western Europe	Denmark	1995-2003	Population Survey	0.0051		Cohort	Prospective	Lohse	2005	[140]

Appendix B

Ethics Agreements

UCL RESEARCH ETHICS COMMITTEE
GRADUATE SCHOOL OFFICE

Dr Kholoud Porter
MRC Clinical Trials Unit
Aviation House
125 Kingsway
London
WC2B 6NH

23 March 2012

Dear Dr Porter

Notification of Ethical Approval
Ethics Application: 3709/001: An international study to characterise recently acquired HIV infection in Estonia, Poland and the Ukraine

I am pleased to confirm that in my capacity as Chair of the UCL Research Ethics Committee I have approved your study for the duration of the project i.e. until December 2015.

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form'.

The form identified above can be accessed by logging on to the ethics website homepage: http://www.grad.ucl.ac.uk/ethics/ and clicking on the button marked 'Key Responsibilities of the Researcher Following Approval'.

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

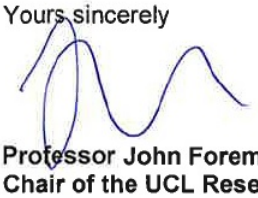
Reporting Non-Serious Adverse Events
For non-serious adverse events you will need to inform Helen Dougal, Ethics Committee Administrator (ethics@ucl.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

Reporting Serious Adverse Events
The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. The adverse event will be considered at the next Committee meeting and a decision will be made on the need to change the information leaflet and/or study protocol.

On completion of the research you must submit a brief report (a maximum of two sides of A4) of your findings/concluding comments to the Committee, which includes in particular issues relating to the ethical implications of the research.

With best wishes for the research.

Yours sincerely



Professor John Foreman
Chair of the UCL Research Ethics Committee

Cc: Ruth Smith

Amendment Approval Request Form

1	**Project ID Number:** 3709/001	**Name and Address of Principal Investigator:** Prof Kholoud Porter MRC Clinical Trials Unit Aviation House 125 Kingsway London WC2B 6NH
2	**Project Title:** An international study to characterise recently acquired HIV infection in Estonia, Poland and Ukraine	
3	**Type of Amendment/s (tick as appropriate)** ☒ Research procedure/protocol (including research instruments) ☒ Participant group ☐ Sponsorship/collaborators ☐ Extension to approval needed (extensions are given for one year) ☐ Information Sheet/s ☐ Consent form/s ☐ Other recruitment documents ☐ Principal researcher/medical supervisor* ☐ Other * *Additions to the research team other than the principal researcher, student supervisor and medical supervisor do not need to be submitted as amendments but a complete list should be available upon request.	
4	**Justification** (give the reasons why the amendment/s are needed) The following modifications have been made to the project 1. Region of focus has changed from Odessa to Kiev. Following the Ukraine parliamentary election in October 2012, the Central Virology and Immunology Laboratory was dissolved, and could no longer function as the testing laboratory for the project as a result the project focus was moved to the Kiev region. Existing testing at the Kiev City AIDS centre will be used to identify newly diagnosed individuals. 2. Modify the method for data collection within Kiev, Ukraine. Existing testing services were used within each country, however within the Ukraine data collected at initial consultation was minimal (Identification number, Year of birth, area of residence and reason for test). To collect the data needed to characterise persons newly diagnosed and to estimate incidence an anonymous questionnaire will be completed by the patient during initial consultation. The self-completed anonymous questionnaire should increase participation and enable patients to be more open regarding risk factors for HIV. 3. Inclusion of viral load and date of viral load to data requirements. Following work conducted by the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) Viral Load has been identified as a key variable in the recent infections testing algorithm. This algorithm uses the test for recent infection (TRI) and clinical information to differentiate recent from longstanding infections. Clinical information indicative of a long-standing infection (low CD4, a low or undetectable viral load, treatment status or clinical stage) is used to reclassify long-standing infections which have been classified as recent when using the TRI. 4. Use of Phylogenetic methods to characterise HIV transmission. Transmission clusters will be investigated using sampled gene sequence data. By investigating transmission clusters we aim to further define the distribution of the HIV epidemic in Ukraine, addressing underreporting of HIV risk due to high levels of stigma and discrimination, particularly among gay men.	
5	**Details of Amendments** (provide full details of each amendment requested, state where the changes	

	have been made and attach all amended and new documentation) The following modifications have been made to the project 1. Region of focus has changed from Odessa to Kiev. Existing testing at the Kiev City AIDS centre will be used to identify newly diagnosed individuals. Please see section 5.2.3 of the attached protocol. 2. Modify the method for data collection within Kiev, Ukraine. An anonymous questionnaire, expanding questions already asked during initial consultation will be introduced. The questionnaire will first be completed by the medical worker entering patient information including, Identification number, Year of birth, Sex and the date of consultation. The questionnaire is then handed to the patient who is given time to review the supporting information accepting whether they are willing to complete the questionnaire. If the patient consents, they answer questions regarding residence, reason for test, risk and testing history. If the patient answers no, the electronic device is handed back to the medical worker. No personal information will be requested and consent will be gained by accepting the completion of the questionnaire. All questionnaires will be completed electronically by the patients and stored using a secure web-based surveillance tool; clinic staff will not have access to patient's questionnaires. Please see section 5.2.3 of the attached protocol and appendix 3. 3. Inclusion of viral load and date of viral load to data requirements. Viral load and date of viral load has been added to the protocol as a data requirement. Please see section 5.3 of the attached protocol. 4. Use of Phylogenetic methods to characterise HIV transmission. All stored residual samples from confirmed HIV positive persons will be sequenced, including historical samples and those collected as part of this study. Samples and data will be collected using methods described in section 5 or for historical samples demographic information on patients will be collated for those who took up a referral for care from the relevant centres. Before analysis all identifiable information will be removed from data to ensure results cannot be linked to patients. Please see section 4.5 and 6.0 of the attached protocol. 5. Ethics approval has been given by all three countries. Please see protocol appendix.
6	**Ethical Considerations** (insert details of any ethical issues raised by the proposed amendment/s)
7	**Other Information** (provide any other information which you believe should be taken into account during ethical review of the proposed changes) Ethics approval has been given by all three countries

Declaration (to be signed by the Principal Researcher)

- I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.
- I consider that it would be reasonable for the proposed amendments to be implemented.
- For student projects I confirm that my supervisor has approved my proposed modifications.

Signature:

Date:

FOR OFFICE USE ONLY:

Amendments to the proposed protocol have been ...approved... by the Research Ethics Committee.

Signature of the REC Chair, Professor John Foreman: .....

Date: 17/7/2013

Tartu Ülikooli inimuuringute eetika komitee

Research Ethics Committee of the University of Tartu (UT REC)

Protokolli number: 216/T-11 **koosolek: 25.06.2012**

Vastutav uurija (asutus):
Irja Lutsar (Tartu Ülikool, Mikrobioloogia Instituut, Ravila 19, Tartu 50411)

Uurimistöö nimetus:
Rahvusvaheline uurimistöö varajase HIV infektsiooni leviku hindamiseks Eestis, Poolas ja Ukrainas 2012-2013

Komitee koosseis:

	Asutus, amet	Osalemine otsuse tegemisel
Aime Keis	TÜ arstiteaduskond, lektor	+
Arvo Tikk	TÜ arstiteaduskond, emeriitprofessor	-
Naatan Haamer	TÜ naistekliinik, hingehoidja	+
Külli Jaako	TÜ arstiteaduskond, vanemassistent,teadur	+
Ruth Kalda	TÜ arstiteaduskond, professor	+
Kaia Kastepõld-Tõrs	TÜ sotsiaal- ja haridusteaduskond, lektor	+
Kristi Lõuk	TÜ filosoofiateaduskond, projektijuht	-
Anu Masso	TÜ sotsiaal- ja haridusteaduskond, lektor	-
Vallo Olle	TÜ õigusteaduskond, dotsent	-
Maire Peters	TÜ naistekliinik, teadur	+
Mare Remm	Tartu Tervishoiu Kõrgkool, dotsent	+
Oivi Uibo	TÜ arstiteaduskond, dotsent	-
Vahur Ööpik	TÜ kehakultuuriteaduskond, professor	+

Komiteele läbivaatamiseks esitatud dokumendid:

1. Uurimistöö avaldus kooskõlastuse saamiseks Tartu Ülikooli inimuuringute eetika komiteelt, täiendatud seisuga 09.07.2012
2. Uurimismeeskonna CV-d (I. Lutsar; P. Soodla)
3. Terviseameti kooskõlastus projektis osalemiseks

Komitee otsus: Luba antud uurimistööks.

Uuringu lõpp: Juuli 2014

Komitee esimees: Aime Keis

Komitee vastutav sekretär: Helen Riismaa

Väljastatud: 12.07.2012

University of Tartu
Office of Research and Development
Ülikooli 18
50090, Tartu, Estonia

Phone: (+372) 7 375 514
Fax: (+372) 7 375 508

KOMISJA BIOETYCZNA
przy
Narodowym Instytucie Zdrowia Publicznego -
Państwowym Zakładzie Higieny
ul. Chocimska 24, 00-791 Warszawa

Komisja Bioetyczna
przy
Narodowym Instytucie Zdrowia Publicznego
Państwowym Zakładzie Higieny
ul. Chocimska 24, 00-791 Warszawa, tel. (022) 5421261

Aneks z dnia 16.03.2012 do opinii Nr 3/2007

W imieniu Komisji Bioetycznej przy NIZP-PZH wyrażam zgodę na przedłużenie badań do roku 2012 prowadzonych w ramach projektu naukowego zgłoszonego przez Prof. dr hab. Andrzeja Zielińskiego i Dr n. med. Magdalenę Rosińską pt. **"Wykrywanie i długofalowa obserwacja nowych przypadków zakażeń HIV w Europie Środkowej i Wschodniej (część projektu CASCADE prowadzona na terenie Polski)"**

Na posiedzeniu Komisji Bioetycznej przy Narodowym Instytucie Zdrowia Publicznego – Państwowym Zakładzie Higieny dnia 29.03.2007r. (Opinia Nr 3/2007 z dnia 29.03.2007r.), projekt ten został zaopiniowany pozytywnie. Zgłaszający wniosek, deklarują kontynuację badań wg tej samej metodyki (tego samego protokołu). Zmianie ulega jedynie nazwa projektu (z CASCADE na EuroCoord), co nie jest przedmiotem oceny Komisji.

PRZEWODNICZĄCY
Komisji Bioetycznej przy
Narodowym Instytucie Zdrowia Publicznego -
Państwowym Zakładzie Higieny
Dr n. med. Tomasz Chmielewski

ВИСНОВОК

Комітету з питань медичної етики ДУ «Інститут епідеміології та інфекційних хвороб ім. Л.В. Громашевського НАМН України»
від 12.02.2013 р.

Розглянувши матеріали запланованого проекту «Міжнародне дослідження з метою визначення випадків недавно придбаної ВІЛ-інфекції в Естонії, Польщі й Україні», наданих програмою Eurocoord (мережа передових науково-дослідних центрів, які ведуть роботу по когортах ВІЛ-інфікованих у Європі):

- ✓ заяву з проханням проведення етичної експертизи матеріалів дослідження;
- ✓ протокол запланованого проекту «Міжнародне дослідження з метою визначення випадків недавно придбаної ВІЛ-інфекції в Естонії, Польщі й Україні»;
- ✓ зразок поінформованої згоди пацієнта;
- ✓ анкета щодо поведінки пацієнта;

дійшли висновку:

- ✓ супроводжувальна документація надана в достатньому обсязі та не містить пунктів, які можуть спричинити порушення медико-етичних норм при проведенні дослідження;
- ✓ відповідно до розділу III п. 3.3. та розділу VIII наказу Державної інспекції з контролю якості лікарських засобів МОЗ України від 14.05.2010р. № 56 «Про затвердження Правил проведення клінічних випробувань медичної техніки та виробів медичного призначення і Типового положення про комісію з питань етики".

Комітет з питань медичної етики ДУ «Інститут епідеміології та інфекційних хвороб ім. Л.В. Громашевського НАМН України» вважає, що подані матеріали дослідження запланованого проекту «Міжнародне дослідження з метою визначення випадків недавно придбаної ВІЛ-інфекції в Естонії, Польщі й Україні» відповідають положенням з питань медичної етики МОЗ України № 281 від 01 ЛІ.2002р.

Голова комітету, д.мед.н., проф. Поліщук О.І.

Секретар, к.мед.н. Покас О.В.

Державна установа
"ІНСТИТУТ ЕПІДЕМІОЛОГІЇ ТА ІНФЕКЦІЙНИХ ХВОРОБ
ім. Л.В. Громашевського
Національної академії медичних наук України"
Учений секретар
"ПІДПИС ЗАСВІДЧУЮ"
Підпис
20 р.

ВИСНОВОК

Комітету з питань медичної етики ДУ «Інститут епідеміології та інфекційних хвороб ім. Л.В. Громашевського НАМН України»

від 31.03.2014р.

Розглянувши матеріали запланованого проекту „Міжнародне дослідження з метою визначення недавно придбаною ВІЛ-інфекції в Естонії, Польщі і Україні" за допомогою новинної сучасної технології з використанням алгоритму RITA:

- Заяву з проханням проведення етичної експертизи матеріалів дослідження;
- протокол запланованого проекту „Міжнародне дослідження з метою визначення недавно придбаною ВІЛ-інфекції в Естонії, Польщі і Україні", який включає в себе в тому числі:
  - Проведення філогенетичного аналізу збудника ВІЛ-інфекції в отриманих зразках крові,
  - Встановлення специфічності тесту нещодавньої сероконверсії ВІЛ з використанням зразків крові, отриманих від пацієнтів;
- зразок проінформованої згоди пацієнта;
- анкета щодо поведінки пацієнта,

Дійшли висновку:

1. Супроводжувальна документація надана в достатньому обсязі та не містить пунктів, які можуть спричинити порушення медико-етичних норм при проведенні дослідження;

2. Відповідно до розділу III п. 3.3., розділу VIII наказу МОЗ України від 03.08.2012 р. № 616 «Про затвердження Правил проведення клінічних випробувань медичної техніки та виробів медичного призначення і Типового положення про комісію з питань етики»

Комітет з питань медичної етики ДУ «Інститут епідеміології та інфекційних хвороб ім.Л.В.Громашевського НАМН України» вважає, що подані матеріали дослідження „Міжнародне дослідження з метою визначення недавно придбаною ВІЛ-інфекції в Естонії, Польщі і Україні" відповідають положенням з питань медичної етики МОЗ України № 281 від 01.11.2002р.

Голова комітету, д.мед.н. Шагінян В.Р.

Секретар, к.мед.н. Покас О.В.

Державна установа
"ІНСТИТУТ ЕПІДЕМІОЛОГІЇ ТА ІНФЕКЦІЙНИХ ХВОРОБ
ім. Л.В. Громашевського
Національної академії медичних наук України"
Учений секретар
"ПІДПИС ЗАСВІДЧУЮ"
Підпис
"____" _____ 20__ р.

Appendix C

Clinical Report Forms

ЗАТВЕРДЖЕНО
Наказ Міністерства охорони здоров'я України
21.12.2010 № 1141

Міністерство охорони здоров'я України		МЕДИЧНА ДОКУМЕНТАЦІЯ Форма первинної облікової документації № 249-7/0 **ЗАТВЕРДЖЕНО** **Наказ МОЗ** _____№_____
(найменування та місцезнаходження закладу, в якому заповнена форма)		
Ідентифікаційний код ЄДРПОУ		

Направлення на проведення дослідження на наявність антитіл до ВІЛ

№ з/п	П.І.Б. або індивідуальний номер особи	Стать	Рік народження	Код обстеження контингентів	Дата взяття крові
1	2	3	4	5	6

Медичний працівник,
який оформлював направлення _____ _____
(посада, прізвище, ініціали) (підпис)

«____»_____ 20____ року

Голова Комітету з питань протидії ВІЛ-інфекції/СНІДу та іншим соціально небезпечним хворобам **С.О. Черенько**

Начальник ДЗ «Центр медичної статистики МОЗ» **М.В. Голубчиков**

Рег. № _____

НАПРАВЛЕНИЕ

Дата _____

Ф.И.О. _____

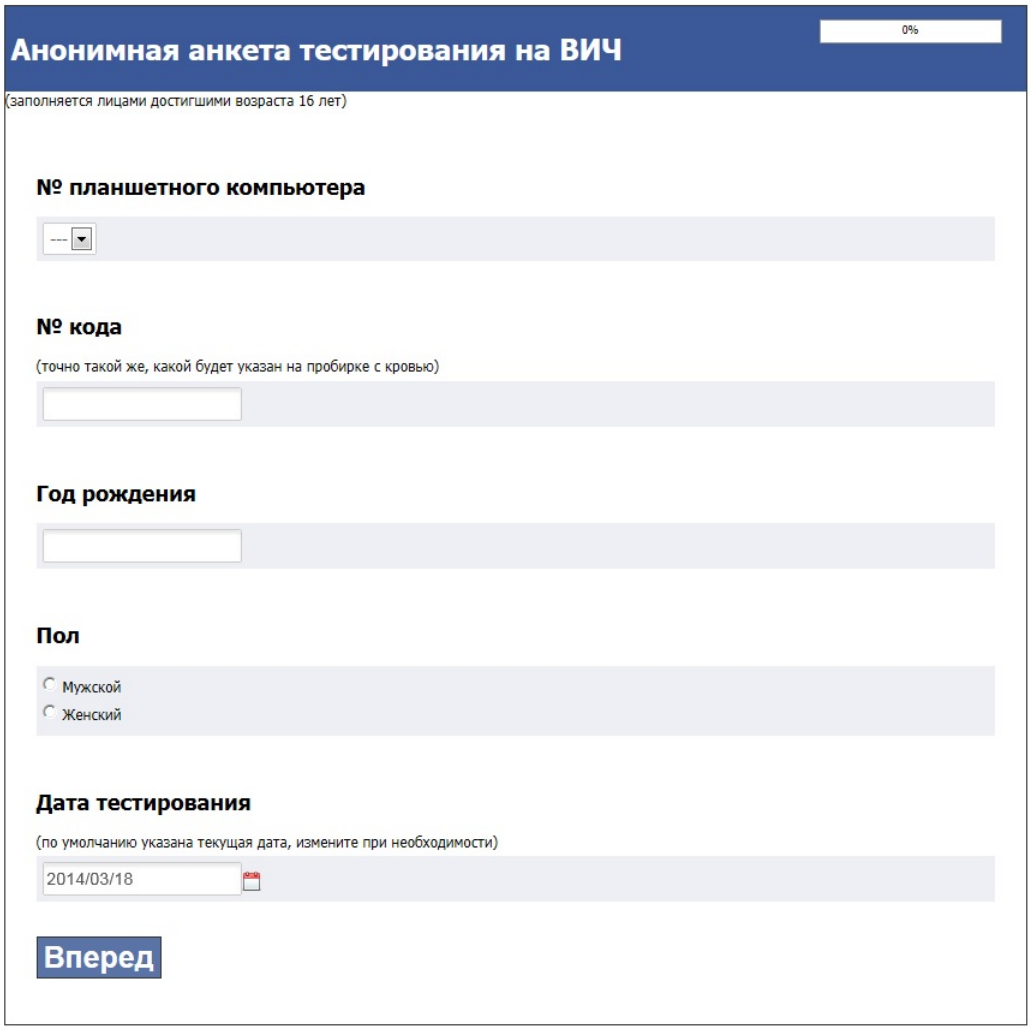
Год рождения _____

Адрес _____

(паспорт) _____

Анализы:	Протокол		
	результат	дата	подпись

Срок выполнения _____


Анонимная анкета тестирования на ВИЧ
0%
(заполняется лицами достигшими возраста 16 лет)
№ планшетного компьютера

№ кода
(точно такой же, какой будет указан на пробирке с кровью)
Год рождения
Пол
Мужской
Женский
Дата тестирования
(по умолчанию указана текущая дата, измените при необходимости)
2014/03/18
Вперед

ИНФОРМИРОВАННОЕ СОГЛАСИЕ

Уважаемый пациент,

Просим Вас уделить 5 минут и заполнить нижеследующую электронную анонимную анкету.

Данное исследование выполняется совместно Киевским НИИ эпидемиологии и инфекционных болезней и Центром медицинских исследований в г. Лондон (Великобритания).

Анкетирование проводиться для того, чтобы лучше понять, какие группы населения подвергаются большему риску заражения ВИЧ. Полученные результаты будут способствовать созданию эффективных мер для замедления темпов распространения эпидемии ВИЧ в стране.

Задаваемые Вам вопросы будут касаться названия города (но не адреса) в котором Вы живете, причин по которым Вы решили сегодня пройти тест на ВИЧ, а также некоторые сведения об опыте предыдущего тестирования на ВИЧ. Пожалуйста, постарайтесь не пропускать вопросы и дайте наиболее полные ответы.

Задаваемые в анкете вопросы имеют исключительно научно-исследовательские цели без малейшего намерения Вас оскорбить или обидеть.

Все Ваши ответы являются полностью анонимными и не могут быть использованы для идентификации Вашей личности. Данные анкет будут доступны только научным исследователям, а не персоналу клиники, где проводилось тестирование на ВИЧ. Полученная информация не будет передаваться третьим лицам, которые могли бы идентифицировать Вашу личность.

Данные анкеты связаны только с результатом теста на ВИЧ. Научные исследователи не будут иметь доступа к Вашим личным данным (Ф.И.О., адрес и др.).

Благодарим Вас за сотрудничество.

Если Вы согласны заполнить данную 5-минутную анонимную анкету, пожалуйста, выберите "Да", подтвердив Ваше согласие.

Согласны ли Вы ответить на вопросы анкеты?

- ○ Да
- ○ Нет

Назад Вперед

Укажите, пожалуйста, Ваше постоянное место жительства

(выберите один наиболее подходящий ответ)

- ○ Киев (город)
- ○ Киевская область (малые города)
- ○ Киевская область (село)
- ○ Другая область Украины
- ○ За рубежом Украины

Укажите, пожалуйста, причины по которым Вы проходите тест на ВИЧ

(выберите из списка все, что подходит)

- ☐ У меня есть симптомы, которые меня беспокоят
- ☐ Инъекционное употребление наркотиков
- ☐ Большое число половых партнеров
- ☐ Контакт с ВИЧ-инфицированным лицом
- ☐ Травматическое повреждение кожи иглой
- ☐ Наличие инфекции передаваемой половым путем
- ☐ Я подозреваю, что у меня ВИЧ инфекция
- ☐ Беременность
- ☐ Получение справки, разрешения и тд
- ☐ Перед хирургическим вмешательством
- ☐ Другое, пожалуйста укажите

Укажите, пожалуйста, были ли когда-либо у Вас нижеперечисленные практики?

(ответьте на каждый из вопросов "да" или "нет")

	Да	Нет
Инъекционное употребление наркотиков	○	○
Половой контакт с лицом противоположного пола	○	○
Половой контакт с лицом одного и того же пола	○	○
Оплачивали сексуальные услуги	○	○
Получали плату или другое материальное вознаграждение за сексуальные услуги	○	○
Половой контакт с лицом употребляющим инъекционные наркотики	○	○

Укажите, пожалуйста, число Ваших половых партнеров за последние 12 месяцев?

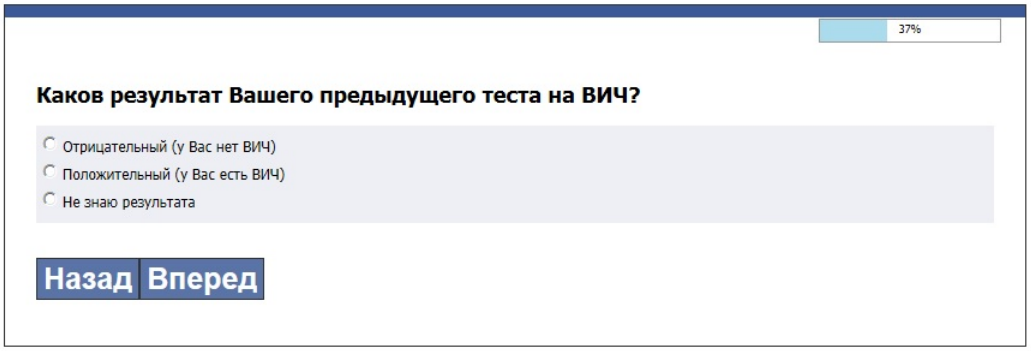
(выберите один наиболее подходящий ответ)

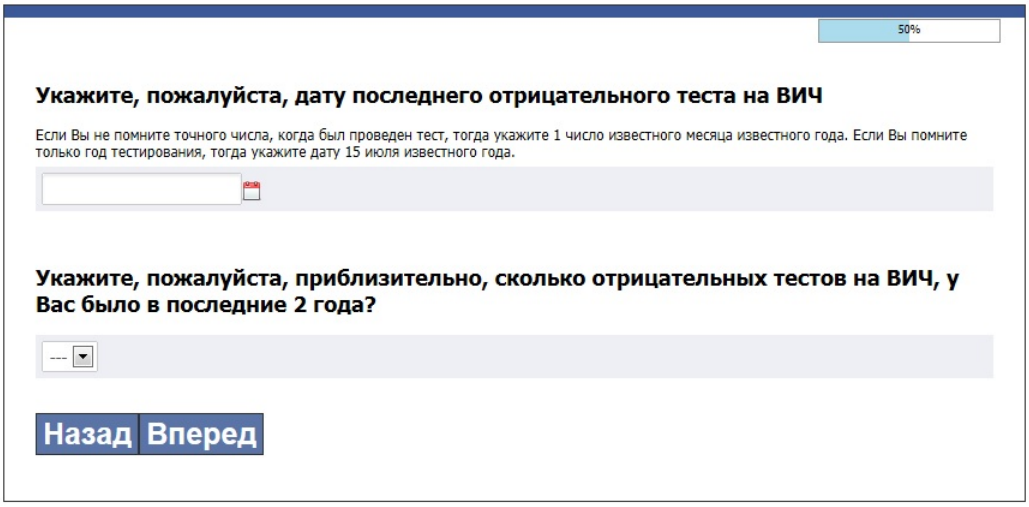
- ○ 1
- ○ 2
- ○ 3-5
- ○ 6-10
- ○ больше 10
- ○ у меня не было половых партнеров в последние 12 месяцев

Приходилось ли Вам когда-либо раннее сдавать тест на ВИЧ?

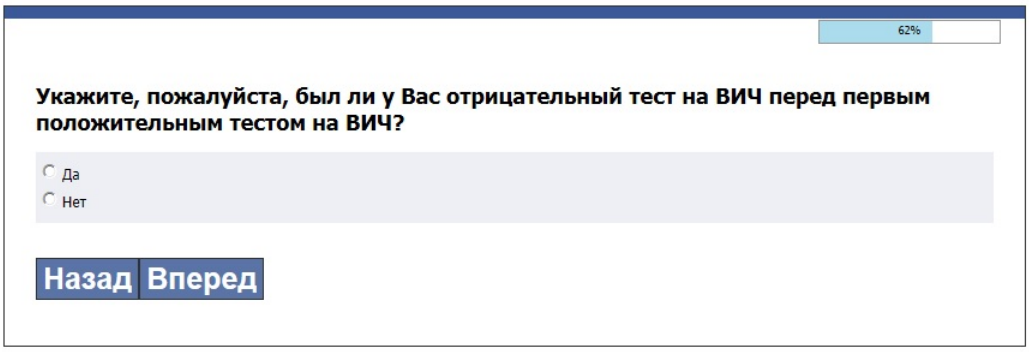
- ○ Да
- ○ Нет

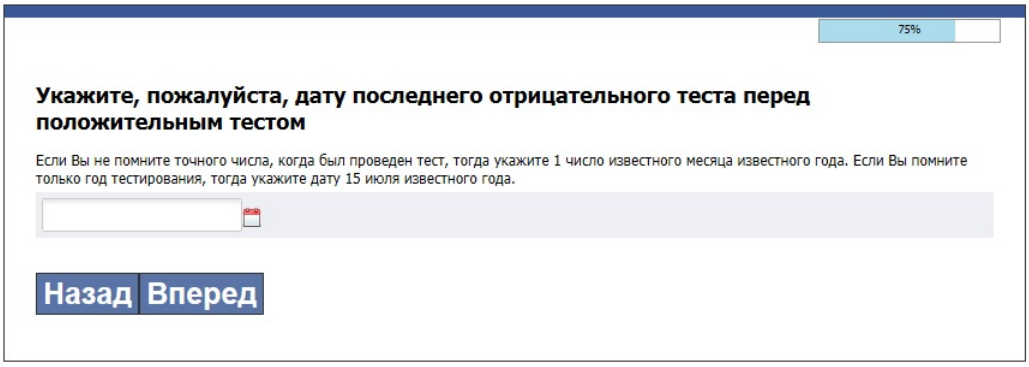
Назад Вперед

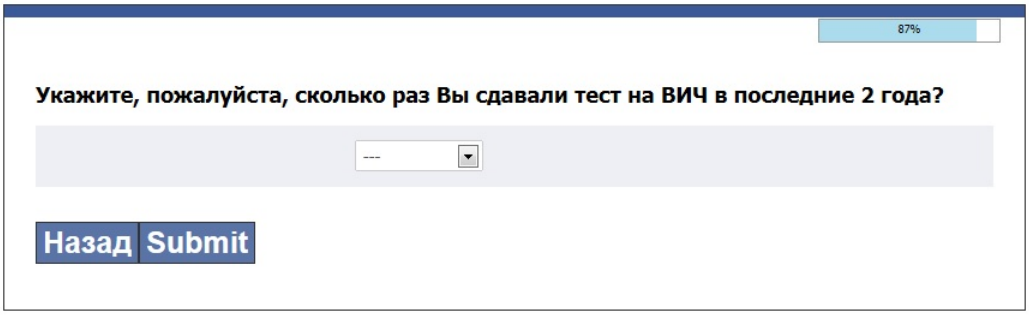

37%
Каков результат Вашего предыдущего теста на ВИЧ?
Отрицательный (у Вас нет ВИЧ)
Положительный (у Вас есть ВИЧ)
Не знаю результата
Назад
Вперед


50%
Укажите, пожалуйста, дату последнего отрицательного теста на ВИЧ
Если Вы не помните точного числа, когда был проведен тест, тогда укажите 1 число известного месяца известного года. Если Вы помните только год тестирования, тогда укажите дату 15 июля известного года.
Укажите, пожалуйста, приблизительно, сколько отрицательных тестов на ВИЧ, у Вас было в последние 2 года?

Назад
Вперед


62%
Укажите, пожалуйста, был ли у Вас отрицательный тест на ВИЧ перед первым положительным тестом на ВИЧ?
Да
Нет
Назад
Вперед


75%
Укажите, пожалуйста, дату последнего отрицательного теста перед положительным тестом
Если Вы не помните точного числа, когда был проведен тест, тогда укажите 1 число известного месяца известного года. Если Вы помните только год тестирования, тогда укажите дату 15 июля известного года.
Назад
Вперед


87%
Укажите, пожалуйста, сколько раз Вы сдавали тест на ВИЧ в последние 2 года?

Назад
Submit

Pieczątka PKD

PŁEĆ: ☐ kobieta ☐ mężczyzna ☐ trans | ROK URODZENIA: | NARODOWOŚĆ: | .....20.....

Miejsce zamieszkania: ☐ miasto ☐ wieś | WOJEWÓDZTWO: | POWIAT:

TEST W KIERUNKU HIV W PRZESZŁOŚCI ☐ NIE ☐ TAK ILE RAZY? ..... KIEDY ostatni? ..... WYNIK: ..... GDZIE? ☐ PKD ☐ INNE jakie? .....

POWTÓRZENIE TESTU PO OKNIE SEROLOGICZNYM? ☐NIE ☐TAK **KRWIODAWCA:** ☐NIE ☐TAK Data ostatniego oddania: miesiąc/rok .....

KONTAKTY RYZYKOWNE (w ocenie doradcy) ☐SEKS ☐IDU ☐ZAWODOWE ☐INNE, jakie? ..... KIEDY? ☐DNI ..... ☐TYGODNIE ..... ☐3-12 M-CY ☐>12 M-CY ☐O.O.

ORIENTACJA SEKSUALNA (deklarowana) ☐HETERO ☐HOMO ☐BISEX ☐O.O. **KONTAKTY SEKSUALNE** ☐M+K ☐M+M ☐K+K ☐BRAK ☐O.O.

RODZAJE KONTAKTÓW SEKSUALNYCH ☐WAGINALNE ☐ANALNE PASYWNE ☐ANALNE AKTYWNE ☐ORALNE PASYWNE ☐ORALNE AKTYWNE ☐INNE, jakie? ..... ☐NIE DOTYCZY ☐O.O.

PARTNERZY SEKSUALNI (ogólnie) ☐K ☐M ☐HETERO ☐HOMO ☐BISEX ☐HIV+ ☐IDU ☐MAJĄCY WIELU PARTNERÓW SEKSUALNYCH ☐SEX WORKERS ☐OBCOKRAJOWCY (kraj?) ..... ☐NIE WIEM ☐NIE DOTYCZY ☐O.O.

LICZBA ☐1 ☐2-5 ☐6-10 ☐11-20 ☐21-50 ☐51-100 ☐101-500 ☐>500 ☐NIE WIEM ☐NIE DOTYCZY ☐O.O.

PARTNERZY SEKSUALNI (ostatni rok) **STALI** ☐K ☐M **LICZBA** ☐0 ☐1 ☐2-5 ☐>5 ☐NIE DOTYCZY ☐O.O. **INNI** ☐K ☐M **LICZBA** ☐0 ☐1 ☐2-5 ☐6-10 ☐11-20 ☐21-50 ☐51-100 ☐NIE DOTYCZY ☐O.O.

STOSOWANIE PREZERWATYW ZE STAŁYM PARTNEREM (ostatni rok) **K.WAGINALNE** ☐ZAWSZE ☐CZĘSTO ☐CZASAMI ☐NIGDY ☐NIE DOTYCZY ☐O.O. **K.ANALNE** ☐ZAWSZE ☐CZĘSTO ☐CZASAMI ☐NIGDY ☐NIE DOTYCZY ☐O.O. **K.ORALNE** ☐ZAWSZE ☐CZĘSTO ☐CZASAMI ☐NIGDY ☐NIE DOTYCZY ☐O.O. **USZKODZENIE PREZERWATYWY** ☐NIE ☐TAK, kiedy? ..... ☐NIE WIEM ☐NIE DOTYCZY ☐O.O.

STOSOWANIE PREZERWATYW Z INNYMI PARTNERAMI (ostatni rok) **K.WAGINALNE** ☐ZAWSZE ☐CZĘSTO ☐CZASAMI ☐NIGDY ☐NIE DOTYCZY ☐O.O. **K.ANALNE** ☐ZAWSZE ☐CZĘSTO ☐CZASAMI ☐NIGDY ☐NIE DOTYCZY ☐O.O. **K.ORALNE** ☐ZAWSZE ☐CZĘSTO ☐CZASAMI ☐NIGDY ☐NIE DOTYCZY ☐O.O. **USZKODZENIE PREZERWATYWY** ☐NIE ☐TAK, kiedy? ..... ☐NIE WIEM ☐NIE DOTYCZY

NARKOTYKI W INIEKCJI ☐NIE ☐TAK ☐O.O. **WSPÓLNE STRZYKAWKI/IGŁY** (nie tylko IDU) ☐NIE ☐TAK, kiedy? ..... ☐O.O.

NARKOTYKI INNE ☐NIE ☐TAK, kiedy? ..... jakie? ..... jak stosowane? ..... ☐O.O.

SEKS POD WPŁYWEM w stopniu zmieniającym świadomość i racjonalne podejmowanie decyzji (ostatni rok) **Narkotyków** ☐NIE ☐TAK ☐O.O. **Alkoholu** ☐NIE ☐TAK ☐O.O. Inne, jakie? .....

TATUAŻ ☐NIE ☐TAK, kiedy? ..... ☐O.O. **PIERCING** ☐NIE ☐TAK, kiedy? ..... ☐O.O. **INNE** .....

STI ☐NIE ☐NIE WIEM ☐TAK, jakie? ..... Kiedy? ..... ☐O.O **WZW B** ☐NIE ☐TAK ☐NIE WIEM ☐SZCZEPIENIE ☐O.O. **WZW C** ☐NIE ☐TAK ☐NIE WIEM ☐O.O.

CIĄŻA ☐NIE ☐TAK, który tydzień? ..... ☐NIE WIEM ☐NIE DOTYCZY ☐O.O. **SZCZEPIENIE W CIĄGU OSTATNIEGO MIESIĄCA?** ☐NIE ☐TAK, jakie? ..... ☐ O.O.

NARAŻENIA ZAWODOWE? ☐NIE ☐TAK, jakie? .....

CZY OBECNY/STAŁY PARTNER WYKONYWAŁ TEST W KIERUNKU HIV? ☐NIE ☐TAK, kiedy? ..... wynik? ..... ☐NIE WIEM ☐NIE DOTYCZY ☐O.O. **ZALECENIE WYKONANIA TESTU U PARTNERA** ☐NIE ☐TAK

ZALECONO WYKONANIE TESTU ☐ NIE, powód? .....

ZALECONO POWTÓRZENIE TESTU ☐NIE ☐TAK, powód? .....

podpis doradcy zalecającego badanie

	−	−/+	+	
WYNIK SZYBKIEGO TESTU				DATA ..... PODPIS .....
WYNIK TESTU PRZESIEWOWEGO				DATA ..... PODPIS .....
WYNIK TESTU POTWIERDZENIA				DATA ..... PODPIS .....

☐Pacjent został pouczony o obowiązku poinformowania partnera seksualnego (partnerów) o swoim zakażeniu oraz o środkach służących zapobieganiu przeniesienia zakażenia na inne osoby i o konieczności zgłoszenia się do lekarza partnera lub partnerów seksualnych zakażonego (zgodnie z art. 26 ust. 1 i 2 Ustawy o zapobieganiu oraz zwalczaniu zakażeń i chorób zakaźnych u ludzi (Dz.U.08.234.1570).

DATA ..... PODPIS doradcy wydającego wynik potwierdzenia .....

PACJENT ROZKODOWAŁ SIĘ ☐TAK ☐NIE

WYNIK NIE ZOSTAŁ ODEBRANY ☐ DATA ..... PODPIS .....

numer badania

hasło

* O.O. - odmowa odpowiedzi

Tu wklej numer badania

ZALECENIE BADANIA W KIERUNKU HIV

obowiązujące w punktach konsultacyjno-diagnostycznych

1. Inicjały imienia i nazwiska ☐☐

   lub hasło: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

2. Rok urodzenia: ☐☐☐☐

3. Płeć: mężczyzna ☐ kobieta ☐

4. Obywatelstwo: polskie ☐ inne ☐ (jakie?) .....

5. Województwo właściwe ze względu na miejsce zamieszkania:

   ☐☐ .....

6. Nazwa powiatu właściwego ze względu na miejsce zamieszkania:

   .....

7. Droga zakażenia:

a) ☐ **krwiopochodna:**

☐ ekspozycja zawodowa ☐ ekspozycja pozazawodowa ☐ IDU

b) ☐ **kontakty seksualne:**

☐ hetero ☐ MSM ☐ WSW ☐ bisex

c) ☐ **IDU + sex**

☐ hetero ☐ MSM ☐ WSW ☐ bisex

d) ☐ **wertykalna**

Uwagi dla laboratorium (np. czas ekspozycji, sytuacje lub substancje mogące zafałszować wynik):

.....	.....	.....
Pieczątka PKD	Data	Czytelny podpis doradcy

- MSM – mężczyzna mający seks z mężczyzną (*men who have sex with men*),
- WSW – kobieta mająca seks z kobietą (*women who have sex with women*).

ANKIETA ANONIMOWA

Tu wklej numer badania!

Wypełnienie niniejszej ankiety nie jest obowiązkowe.
Jednakże zwracamy się do Pani/Pana z prośbą o pomoc w uzyskaniu szerszej informacji, która będzie przydatna w tworzeniu programów profilaktycznych.
Będziemy wdzięczni, jeżeli zdecyduje się Pani/Pan na wypełnienie tego krótkiego kwestionariusza.

Serdecznie dziękujemy

Krajowe Centrum ds. AIDS

Wykształcenie: ☐ podstawowe ☐ zawodowe ☐ średnie ☐ wyższe

Status rodzinny: ☐ stanu wolnego ☐ zamężna/żonaty ☐ rozwiedziona/y ☐ związek nieformalny ☐ wdowiec/wdowa

Obecna sytuacja zawodowa: ☐ zatrudniony ☐ bezrobotny ☐ uczeń szkoły średniej ☐ student ☐ rencista/emeryt ☐ inne.....

Ocena własnej sytuacji materialnej: ☐ bardzo dobra ☐ dobra ☐ zadowalająca ☐ zła ☐ bardzo zła

Ocena swojego stanu zdrowia: ☐ bardzo dobry ☐ dobry ☐ średni ☐ zły

Skąd dowiedział(a) się Pan(i) o Punkcie Konsultacyjno–Diagnostycznym (PKD)?

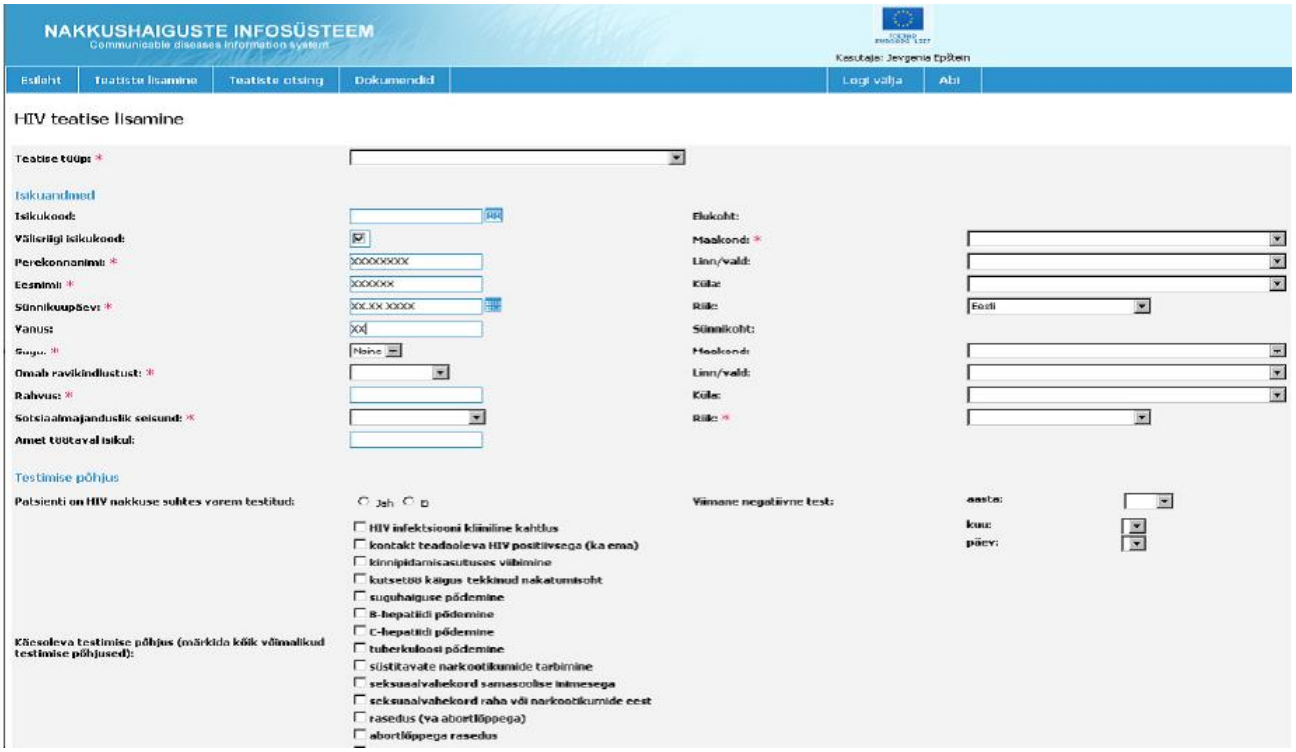
(można zaznaczyć więcej niż jedną odpowiedź)

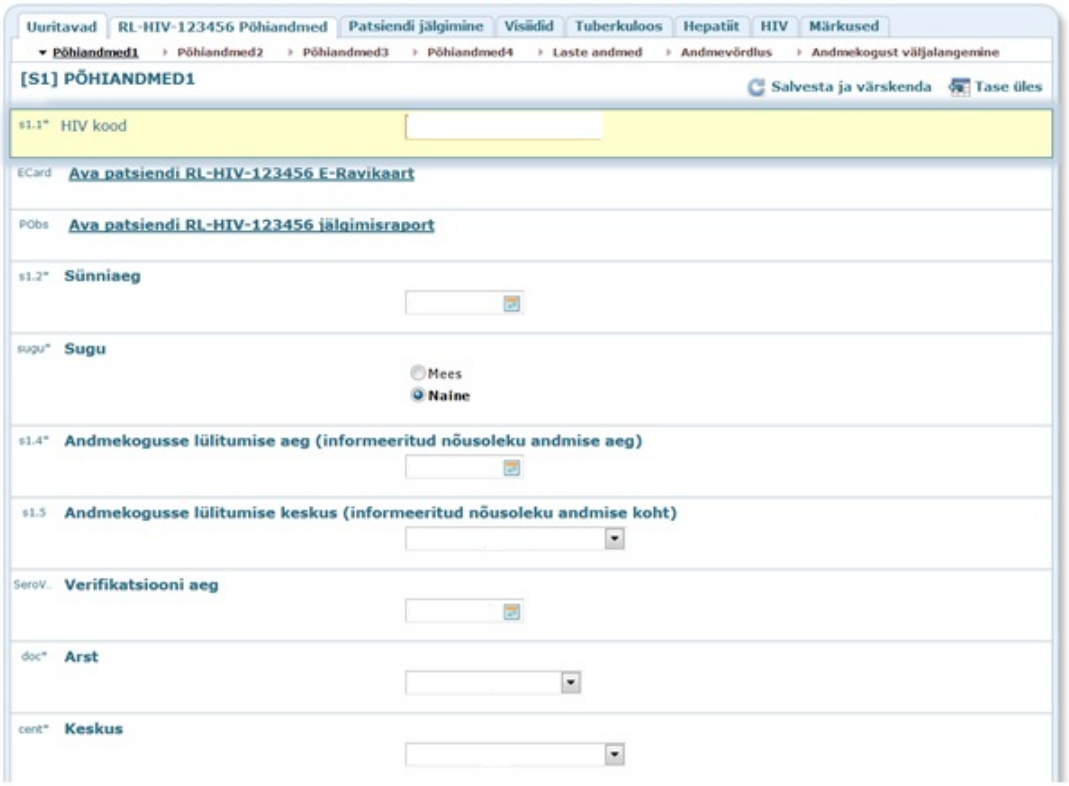
☐ od znajomych ☐ z Internetu ☐ z ulotki
☐ od lekarza ☐ z telefonu zaufania ☐ z kampanii medialnej
☐ od osoby, która wykonała badanie w PKD ☐ inaczej, jak: .....

---

klientowi

Jeżeli jest Pan(i) zainteresowany(a) uzyskaniem dodatkowych informacji o HIV/AIDS oraz wypełnieniem ankiety oceniającej pracę Punktu Konsultacyjno – Diagnostycznego zapraszamy do odwiedzenia strony internetowej www.aids.gov.pl.


NAKKUSHAIGUSTE INFOSÜSTEEM
Communicable diseases information system
Kasutaja: Jevgenia Epštein
Esileht
Teatiste lisamine
Teatiste otsing
Dokumendid
Logi välja
Abi
HIV teatise lisamine
Teatise tüüp: *
Isikuandmed
Isikukood:
Välisriigi isikukood:
Perekonnanimi: *
Eesnimi: *
Sünnikuupäev: *
Vanus:
Sugu: *
Oma ravikindlustust: *
Rahvus: *
Sotsiaalmajanduslik seisund: *
Amet töötaval isikul:
Elukoht:
Maakond: *
Linn/vald:
Küla:
Riik:
Eesti
Sünnikoht:
Maakond:
Linn/vald:
Küla:
Riik: *
Testimise põhjus
Patsienti on HIV nakkuse suhtes varem testitud:
Jah
Ei
Viimane negatiivne test:
aasta:
kuu:
päev:
Käesoleva testimise põhjus (märkida kõik võimalikud testimise põhjused):
HIV infektsiooni kliiniline kahtlus
kontakt teadaoleva HIV positiivsega (ka ema)
kinnipidamisasutuses viibimine
kutsetöö käigus tehtimud nakatumisoht
suguhaiguse põdemine
B-hepatiidi põdemine
C-hepatiidi põdemine
tuberkuloosi põdemine
süstitavate narkootikumide tarbimine
seksuaalvahekord samasoolise inimesega
seksuaalvahekord raha või narkootikumide eest
rasedus (va abortlõppega)
abortlõppega rasedus


Uuritavad
RL-HIV-123456 Põhiandmed
Patsiendi jälgimine
Visiidid
Tuberkuloos
Hepatiit
HIV
Märkused
Põhiandmed1
Põhiandmed2
Põhiandmed3
Põhiandmed4
Laste andmed
Andmevõrdlus
Andmekogust väljalangemine
[S1] PÕHIANDMED1
Salvesta ja värskenda
Tase üles
s1.1* HIV kood
ECard Ava patsiendi RL-HIV-123456 E-Ravikaart
PObs Ava patsiendi RL-HIV-123456 jälgimisraport
s1.2* Sünniaeg
sugu* Sugu
Mees
Naine
s1.4* Andmekogusse lülitumise aeg (informeeritud nõusoleku andmise aeg)
s1.5 Andmekogusse lülitumise keskus (informeeritud nõusoleku andmise koht)
SeroV.. Verifikatsiooni aeg
doc* Arst
cent* Keskus

Appendix D

Published Papers

High percentage of recent HIV infection leading to onward transmission in Odessa, Ukraine associated with young adults

Ruth Smith[1], Igor Semenenko[2], Maria Tolpina[3], Rostislav Tereschenko[4], Ludmila Kotlik[3], Lyubov Zasyptka[3], Gary Murphy[5], Elaine McKinney[5], Ruslan Malyuta[2], Kholoud Porter[1] on behalf of CASCADE Collaboration in EuroCoord

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Background

- By 2009, Ukraine had the highest reported rates of HIV in Europe, with 35.4 cases per 100,000 population.
- To date, it is not clear what proportion of new HIV diagnoses in the Ukraine are new infections. Differentiating between recent and long-standing infection would allow current transmission patterns to be identified, highlighting populations at greater risk and tailoring prevention and intervention strategies.
- Laboratory techniques have been developed to use the maturing antibody response of the infection to identify those with a recent infection. Such techniques are collectively known as the recent infection testing algorithm (RITA). These laboratory assays are a practical alternative to observational studies which are expensive to run as they require follow up of sero-negative populations over long periods of time.
- In this study, we aimed to estimate the proportion of new HIV diagnoses which are recent infection among individuals with new HIV diagnoses in the Ukraine region of Odessa. We used a RITA assay and explored factors associated with testing as a recent infection.

Methods

- All newly-diagnosed HIV positive adults (≥16 years) across Odessa city and province between May and December 2009 were included.
- The following information was collected: basic demographic information such as name, year of birth, residence, and reason for test. No information was available on previous AIDS diagnosis or prior use of ART. However, HIV positive individuals were referred to a clinical centre for HIV/AIDS, available in each province, where HIV care is provided. Further information on HIV disease stage was available for individuals who took up the referral.
- Residual samples were tested for evidence of recent infection using the BED-CEIA assay.
- Logistic regression models were used to investigate the effect of age, sex, reason for test, residence (city, rural, non-resident) on testing recent.

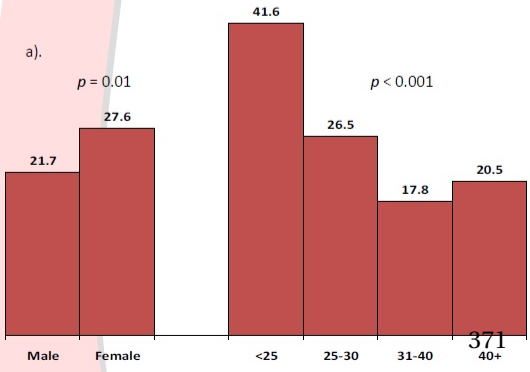
Results

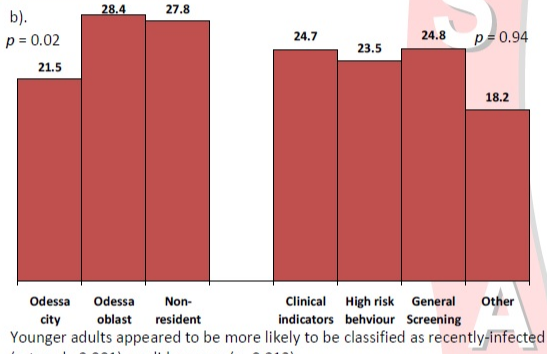
Demographic characteristics and indication for HIV test (N=1313)

		New HIV diagnoses
Age at HIV diagnosis (years)	<25	209
	25-30	343
	31-40	488
	40+	273
Sex	Male	709
	Female	604
Residence	Odessa: City	692
	Odessa: Rural	550
	Non-resident in Odessa	54
	Not reported	17
Reason for HIV test	Clinical indicators	477
	High risk behaviour	268
	General public screening	557
	Other	11

- Of 1,315 persons aged ≥16 years tested with the BED assay, 2 were excluded as they were subsequently discovered to be HIV negative.
- Of the remaining 1,313, 321 (24%) were classified as recent.
- Median age at HIV diagnosis was 32 years [inter quartile range (IQR): 26-39], with men accounting for 54% of diagnoses.
- The majority of persons tested for HIV were part of general public screening (42%) or due to clinical indications (36%)
- By May 2011, 819 (62%) of those newly diagnosed had presented at an HIV/AIDS centre.

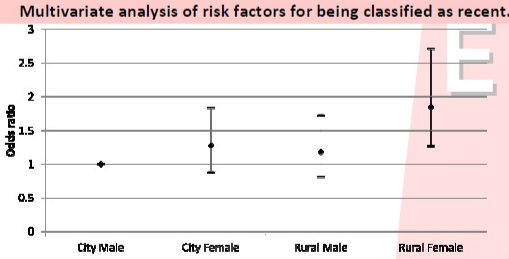
Percentage testing recent (N=321) of total newly diagnosed (N1313) a). By sex and age b). By residence and indication for testing.





- Younger adults appeared to be more likely to be classified as recently-infected (p-trend <0.001), as did women (p=0.013).
- Those resident in the city of Odessa were less likely to be classified as recently-infected (p=0.018).
- The interaction between sex and residence was found to be significant (p=0.015), with women residing in rural Odessa more likely to be recent (p=0.008) compared to men in Odessa city.
- Information on clinical status was available for 200 of the 321 classified as recently infected, of which 16% had an AIDS diagnosis and 11% had died. After removing all individuals tested due to clinical indications the logistic regression analysis remained qualitatively unchanged.

Multivariate analysis of risk factors for being classified as recent.



Conclusions

- One in four persons with a new HIV diagnosis in Odessa in 2009, were classified as recently-infected, suggesting that the risk of onward transmission is high
- A high proportion of individuals were misclassified as recently-infected with an AIDS or died within 2 years of diagnosis. After excluding individuals testing due to clinical infections predictors for testing as a recent infection remained unchanged.
- Age was found to be an independent risk factor for testing as recent, with a higher probability with younger age.
- Women resident in the city and rural Odessa were more likely to be classified as recent than their male counter parts.

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