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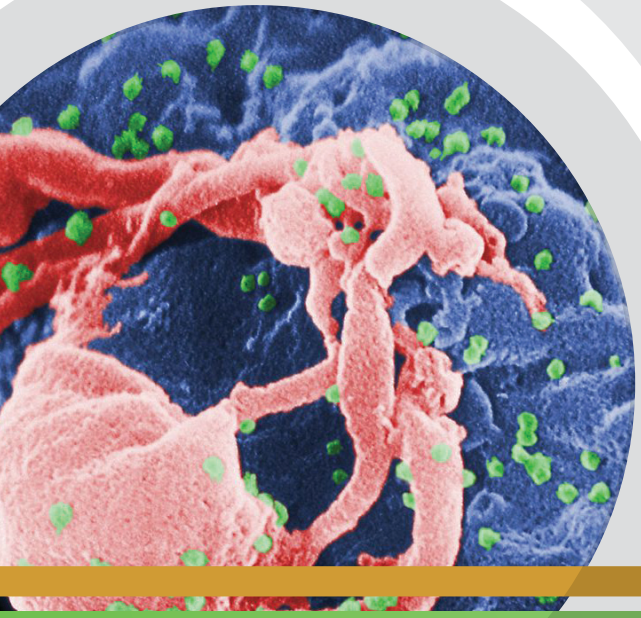

TECHNICAL REPORT

Monitoring recently acquired HIV infections in the European context

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
aOR	Adjusted odds ratio
ART	Antiretroviral treatment
ARV	Antiretrovirals
AST	Anonymous testing site
CEPHIA	Consortium for the Evaluation and Performance of HIV Incidence Assays
CI	Confidence interval
ECDC	European Centre for Disease Control
EEA	European Economic Area
EU	European Union
ART	Antiretroviral therapy
FRR	False-recent rate
HIV	Human immunodeficiency virus
IDU	Injecting drug use
MARP	Most-at-risk population
MSM	Men who have sex with men
MTCT	Mother-to-child transmission
OR	Odd ratio
PHI	Primary HIV Infection
PWID	People who inject drugs
RITA	Recent Infection Testing Algorithm
RI	Recent infection
SOP	Standard operating procedure
STARHS	Serological testing algorithm for recent HIV seroconversion
STI	Sexually transmitted infection
TRI	Test for recent infection
VCT	Voluntary counselling and testing
WHO	World Health Organization

Executive summary

In most EU/EEA countries, HIV epidemics are largely concentrated in subpopulations, namely men who have sex with men, migrants (particularly from countries with a high prevalence in the general population), and people who inject drugs. The introduction of effective antiretroviral therapy (ART) in the mid-1990s resulted in improved life expectancy for people diagnosed with HIV. Consequently, the number of AIDS cases and deaths has declined dramatically in countries where ART is readily available. Longer life expectancy and ongoing transmission has resulted in year-on-year increases in HIV prevalence and high rates of new diagnoses across Europe. The need to better understand the transmission dynamics of the epidemic and monitor prevention efforts in populations most at risk is therefore more critical than ever.

Current approaches to HIV surveillance in Europe rely primarily on the reporting of new diagnoses of HIV and AIDS cases, and on those accessing HIV-related treatment and care services. Existing HIV testing and surveillance methods are unable to distinguish whether this is due to an increase in HIV transmission due to increased testing of undiagnosed infections. Tests to differentiate recent from long-standing infections, initially known as 'serological testing algorithms for HIV seroconversion' and more recently as Recent Infection Testing Algorithms (RITA), have become an increasingly attractive approach to monitoring recent HIV infections and are used for estimating HIV incidence in several countries.

A range of tests for recent infection have been developed over the last decade; each quantifies a different aspect of the natural history of an individual HIV infection and is subject to different intra- and inter-person variability and other biases. Incidence measures derived from these assays used different definitions of a recent infection due to variations in the window period. Despite the rapid development of different tests, there is lack of commercially available test kits and wider evaluation of tests. Therefore currently produced results hamper comparability.

The literature review provides a range of estimates in key populations, highlighting studies that have employed a RITA assay to distinct populations in order to produce estimates of HIV incidence, and pays particular attention to variations in assays, settings, window periods, and methods of analysis. Estimates of HIV incidence across populations most at risk were generally below 1–2% per year, and below 4% per year among attendees of STI clinics and similar high-risk settings. Estimated HIV incidence rates among men who have sex with men were generally less than 5% per year, similar to those reported among migrant populations and ethnic minorities. However incidence among migrant populations varied markedly between ethnic minority group and country of origin. People who inject drugs were not included in many studies, possibly reflecting the fact that the majority of RITA publications were from Western Europe and the United States, where injecting drug use is a less common transmission route for acquiring HIV infection. Among people who inject drugs, the incidence estimates ranged from 0% per year across networks of publically funded counselling and testing sites to approximately 8% per year among STI clinic attendees. Recent infections in young people were generally lower, with the majority of estimates below 3% per year.

Overall, published incidence estimates based on studies using RITA assays were difficult to compare due to a lack of consistency in study design, selected populations, type of assays used, and presentation of the major findings. Furthermore, the sample sizes of the population of interest were often too small and the results unlikely to be generalisable to the overall risk populations.

A framework of HIV incidence studies in Europe was developed taking into account issues highlighted in the literature review and suggests, for the European context, the need to incorporate RITA assays as part of large-scale studies or surveillance schemes to ensure sufficient sample sizes are achieved and the samples included are representative of the populations from which they are drawn. In accordance with the framework, a technical guide on how to integrate RITA as part of routine HIV surveillance has been developed.

The implementation of the framework with the technical guide offers the potential to coordinate and strengthen HIV incidence surveillance activities across European Member States by providing insights into current transmission patterns and dynamics of HIV infection within subpopulations. In addition, these data will guide HIV prevention and health promotion efforts allowing targeted interventions among the subset of persons most at risk of infection.

1 Introduction

1.1 Monitoring recently acquired HIV infections

In most EU/EEA countries, epidemics are largely concentrated in subpopulations, namely men who have sex with men (MSM), migrants (in particular those from countries with a generalised HIV epidemics), and people who inject drugs (PWID) [1] [2] [3]. In 28 EU/EEA countries, 27 116 cases of HIV infection were diagnosed and reported in 2010; a rate of 5.7 per 100 000 population [4]. Among these cases, male-to-male sex was the predominant mode of transmission (38%), followed by heterosexual transmission (24%) (excluding cases from countries with generalised epidemics), injecting drug use (4%), and mother-to-child transmission (1%).

The introduction of effective antiretroviral therapy (ART) has contributed to improved life expectancy for people diagnosed with HIV. Consequently, the number of AIDS cases and deaths declined dramatically in the mid-1990s in countries where ART has been widely available [5]. Longer life expectancy and ongoing transmission has resulted in a year-on-year increase in the prevalent pool of persons living with HIV (PLWH) in specific populations and therefore potentially an increased risk of transmission in these groups. Against a background of increasing numbers of newly diagnosed infections across the EU/EEA, the need to better understand the transmission dynamics of the epidemic and monitor prevention efforts in subpopulations most at risk has become more critical than ever.

Controlling the spread of HIV across Europe requires an understanding of those persons at risk of becoming infected, and the tailoring of key prevention interventions and health promotion initiatives to meet their needs. However, the surveillance and monitoring of the HIV epidemiology pose unique challenges given the extended period between infection and the onset of symptoms, typically between eight and eleven years later, and the concentration of infection in often marginalised populations that are hard to reach. Current approaches to HIV surveillance in Europe rely primarily on the reporting of new diagnoses of HIV and AIDS cases, and on those accessing HIV-related treatment and care services. In recent years it has become increasingly apparent that these approaches are slow to reflect possible changes in HIV transmission and equally slow to show the possible impact of public health interventions on HIV transmission.

Since the development of a number of laboratory techniques, it has been possible to determine among all newly diagnosed cases of HIV, those that are likely to represent recently acquired HIV infections using a single sample [6] [7] [8]. These tests, initially known as Serological Testing Algorithms for HIV Seroconversion (STARHS), and more recently as Recent Infection Testing Algorithms (RITA), have become increasingly appealing for monitoring recent HIV infections, and serve as a basis for estimating the proportion of newly acquired HIV infections and HIV incidence in several European countries (Annex 2). This offers the potential to coordinate and strengthen HIV incidence surveillance activities and provide insights into current transmission patterns and dynamics of the infection within subpopulations across Europe. In addition, these data will guide HIV prevention and intervention strategies, including earlier HIV testing. RITA data will allow health promotion efforts across Europe to specifically focus on the subset of HIV infections that have been recently acquired, and deliver a range of benefits that include a deeper understanding of the transmission of HIV among groups at risk, monitor and evaluate prevention initiatives, target resources to the populations in greatest need, and highlight those programmes that are most effective in reducing transmission. In addition, there exists significant research potential focusing on the behavioural characteristics of those recently infected.

In 2006, following the STARHS symposium held at the XVI International AIDS Conference in Toronto, it was agreed that there was a need to standardise approaches for validating RITA assays. An initial protocol for the calibration and validation of RITA assays was subsequently drafted by the US Centers for Disease Control and Prevention (CDC). The World Health Organization Technical Working Group on HIV Incidence Assays was established in 2008 with the aim of sharing and advancing the science on HIV incidence assays, creating a central resource/knowledge repository, providing guidance and advocacy documents on the use of HIV incidence assays, and assessing and fostering the development of improved HIV incidence assays. A comprehensive literature review on the calibration and validation of HIV incidence assays was also undertaken, supported by the WHO working group, to support the development of the protocol. Further information about the working group and meeting minutes can be found on the WHO HIV incidence webpage at http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/index.html.

1.2 Scope and purpose

This report describes the spectrum of HIV incidence studies relevant for Europe and provides the background for the development of harmonised HIV incidence activities in Europe. It contains the results of a literature review of published incidence studies using RITA assays, an epidemiological framework for HIV incidence studies in Europe, and a technical guide on how to integrate RITA as part of routine HIV surveillance.

Literature review

The review provides a range of estimates in key groups at risk, highlighting studies that have employed a RITA assay to distinct populations. The application of RITA assays in Europe, North America, Canada and Australia to determine either the proportion of recent infections or to estimate HIV incidence is the focus of this review. A brief overview of RITA assays used is summarised in Annex 2. For a full description of the assays and their accuracy, the reader is referred to other relevant reviews [9] [10] [11].

The main objectives were to: identify, through a comprehensive literature search, those papers, reports, abstracts, dissertations, surveillance summaries, public health reports, containing information on HIV incidence studies using RITA assays, which are pertinent to the European context; identify all EU countries that are conducting, or have conducted RITA-based HIV incidence studies; summarise all EU country data on HIV incidence, including any available trends data; and summarise methods used to calculate HIV incidence in the selected studies, and in particular study design and sampling methods.

Although a variety of statistical methods have been used in the published literature to calculate incidence using RITA tests, one of the most frequently used approaches is that described by Janssen [6]. This method has been critiqued in a number of reviews [12] [13] [14]. For more details on RITA tests available, sample size calculations and statistical consideration when planning a RITA study, please refer to the WHO incidence working group document [10].

Epidemiological framework

The findings of the literature review were used in the development of an epidemiological framework for the monitoring of recently acquired infections in the EU/EEA which aims to harmonise HIV incidence approaches using RITA assays across Europe.

The framework of HIV incidence studies in Europe takes into account feedback from HIV incidence experts and addresses several issues highlighted in the literature review. During the implementation of the framework, further HIV incidence activities were presented. The aim was to guide the application of RITA across Europe, therefore the framework provides recommendations on the most appropriate epidemiological study designs and describes statistical and analytical methods to estimate HIV incidence. A key component of a RITA framework is the identification of key clinical, epidemiological and laboratory variables which should form part of the future public health application of RITA. The framework also offers the potential to coordinate and strengthen HIV incidence surveillance activities across Europe.

Technical guide for the implementation of RITA

In accordance with the framework, and with input from experts in France and the UK – the only countries in Europe currently routinely performing recent infection testing on new HIV diagnoses – a technical guide for the implementation of RITA was developed. This guide provides practical information concerning the data requirements and epidemiological, laboratory and statistical aspects of applying RITA to surveillance data. It highlights the caveats regarding the interpretation of outputs which are specific to surveillance data, and suggests how to report these, promoting consistency both in methods applied and the presentation of data.

2 Literature review

A systematic search of PubMed was undertaken using a number of key terms. These terms were used to search MeSH terms, article titles and abstracts (Annex 3). A total of 48 peer-reviewed publications, three conference abstracts and one poster were included in the evidence syntheses, covering the years 1998 to September 2010. Of these, the majority were published in the United States of America (N=26), followed by France (N=4), the United Kingdom (N=3), and the Netherlands (N=3).

Findings are presented in the following sections by key populations, with the recognition that some overlap exists across these populations (for example, MSM can be young *and* from diverse ethnic backgrounds).

2.1 Types of RITA tests

Among 44 studies reviewed, the Vironostika LS-EIA was the most frequently used platform for identifying incident infections (N=20), followed by BED-CEIA (N=13), Abbott 3A11 assay (N=5), IDE-V3 (N=3) and AxSYM Avidity (N=3). There were marked differences in the RITA method by country, with IDE-V3 most common among French studies, BED-CEIA among German and US studies, Vironostika among UK studies, and AxSYM among Italian and Austrian studies. This correlated with where the respective tests were developed. Furthermore, earlier studies, primarily in the US, used the Vironostika LS-EIA in earlier years, switching to the BED-CEIA in recent years. The use of some tests has been largely confined to Europe, in particular the IDE-V3 (France) and AxSYM Avidity tests (Italy, Spain and UK) although these latter tests are now also used outside Europe.

The field of HIV incidence measuring is developing fast, however there is a dearth of commercially available test kits and a lack of test evaluations. Therefore, results are difficult to compare.

2.2 Recently acquired HIV infections in young people

Young people are usually defined as persons aged between 15–25 years although there is considerable variation in this definition among published studies. Several studies, particularly in the US, have reported the incidence of HIV in young people using RITA assays (Annex 4, Figure 1). In New York City, the incidence of HIV was reported from a network of 110 publically funded HIV testing sites across the city in 2001, in which young persons accounted for an estimated 30% of all HIV tests. The annual incidence was 0.19% (CI 0.12–0.26) among those aged 20–24 and 0.10% (CI 0.05–0.15) in those aged under 20 [15]. However, it is important to note that estimates vary widely across studies despite similar study sites. Between 1996–2002, HIV incidence was estimated to be 1.3% per year (one order of magnitude higher than the New York City studies) among young people attending anonymous HIV testing sites in San Francisco – this may be explained by the fact that the population tested in the latter study comprised largely of young MSM [16]. Furthermore, HIV incidence rates were reported to be higher among younger (21–25 years) MSM compared with older MSM, reaching as high as 2.6% per year (95% CI 0.87–6.3) among men attending the same testing sites in a given city between 1996–98 [24]. Other studies do not support these findings and report similar rates of HIV incidence among younger and older MSM [17].

Estimates of HIV incidences were reported to be high among STI attendees. In large HIV incidence studies involving STI clinic attendees in nine US cities conducted between 1991–97, HIV incidence was estimated to be 0.6% per year among those aged 20–29 (0.3% per year among those aged <20) [18]. This increased to 1.3% per year among 20–29-year-olds in follow-up studies conducted across ten US cities between 1997 and 2001 [19].

The incidence of HIV among young people has also been estimated using RITA tests routinely collected on all newly diagnosed individuals across 22 US states. Using statistical formulae that take into account HIV testing patterns, the average annual HIV incident infections among 13–29-year-olds was estimated to be 0.03%, compared with 0.04% and 0.03% among those aged 30–39 and 40–49 years, respectively [20] [21].

The proportion of recent infections among newly diagnosed young persons and subsequently tested with a RITA assay has also been reported in a number of publications (Annex 4, Figure 2). A three-year cross-sectional study involving 28 laboratories serving hospital outpatient clinics, STI clinics and community-based testing sites in Catalonia, Spain, indicated that those under 30 years of age at the time of diagnosis accounted for approximately 37% of all recent infections; this number increased to almost half (49%) in community-based testing sites [22]. In the national surveillance of recent HIV infections in France between 2003 and 2006, 26% of those aged below 30 years of age were diagnosed with a recent infection and accounted for 30% of all recent infections [23]. A similar proportion of recent infections was seen among blood donors: 24% of recently infected HIV-positive persons were younger than 30 years of age [24]. In a multivariate analysis of factors independently associated with recent infection in France between 2003 and 2005, being aged 15–29 carried the highest adjusted OR of 1.52 (CI 1.18–1.97) [25]. In a Los Angeles study of STI clinic attendees, over half of newly diagnosed young men aged 20–29 were likely to have recently acquired their infection, with higher rates among those presenting with early syphilis [26].

Traditionally, trends in HIV prevalence among young people aged 15–24 have been assumed to be an approximation of trends in HIV incidence. This assumption, based upon the fact that the onset of sexual risk behaviour has been more recent among younger individuals, is best illustrated for MSM, the group most at risk of acquiring HIV in western industrialised countries. Between 1989 and 1999, standard and RITA assays conducted among young MSM attending STI clinics in San Francisco (Annex 4, Figure 3) showed a significant decline in HIV prevalence (from 5% to 1.3% (OR 0.84 per year, CI 0.8–0.89)) in parallel with a significant decline in incidence (from 2% per year to 0.32% per year (OR 0.84 per year; CI 0.77–1.0)) [27]. In contrast, no relation was observed among older MSM attending the same sites over the ten-year period [28].

It is important to note that where available, the confidence intervals provided in these studies are very wide, most likely due to the small number of recent infections identified among the tested population. Moreover, several studies reported point estimates only so that the accuracy of the estimate is unknown.

2.3 Recently acquired HIV infections in men who have sex with men

More incidence studies using RITA tests have been conducted among MSM than any other at risk population. Although US studies dominate, there are a substantive number of studies from Europe including France, the UK, Netherlands and Italy, as well as Australia and Canada. Similarly to the studies conducted among young people, many of the studies have sample size limitations (with wide large confidence intervals) or only provide point estimates.

HIV incidence using RITA assays among MSM has been compared with estimates derived from cohort studies. In the Netherlands, HIV incidence estimates among MSM derived from two cohort studies (Amsterdam Cohort Study and Rotterdam Cohort Study) were compared with incidence estimates from RITA testing among MSM attending a STI clinic in Amsterdam [17]. The Amsterdam cohort recruited MSM volunteers through a combination of convenience and chain-referral sampling through community venues and STI clinics, whilst the Rotterdam cohort was recruited by volunteers in community venues such as bars and saunas. In 1999–2005, incidence among STI clinic attendees was 3.75% per year (CI 2.37–5.77), compared with 1.24 (CI 0.89–1.72) and 1.53 (CI 0.82–2.84) in the Amsterdam and Rotterdam Cohort studies, respectively (Annex 4, Figure 4). Between 1991 and 2001, HIV incidence increased among STI clinic attendees from 3.4% per year (CI 0.7–10.2) to 4.4% per year (CI 1.2–11.0), yielding an overall incidence of 3.2% per year (CI 2.0–4.8), however this increase was mainly seen among older MSM aged 40 and over [17] [29]. One study compared estimates derived using RITA assays with those based on either self-reported previous negative, or documented previous negative linked via unique anonymous codes. RITA estimates were consistently higher: 2.1% (CI 1.4–2.8) versus 1.6% (CI 1.5–1.9) and 1.4% (CI 0.9–2.1), respectively [30]. However, another US study reported similar estimates between the RITA and non RITA-based methods [16].

Some studies provided estimates comparing two RITA tests performed on the same specimen. Overall, there was a lack of consistency of performance of the tests across studies, highlighting the need for greater consistency and comparability of tests used. For example, in a study by Truong HM et al. (2000), HIV incidence rates among MSM attending testing sites in San Francisco were measured using both the Vironostika-LS and BED CEIA RITA assays, giving results of 3.7% and 4.0%, respectively [31]. HIV incidence estimates at a local municipal STI clinic in the same year gave estimates of 3.5% and 3.4% respectively using these tests [31].

The majority of studies that used RITA assays to estimate HIV incidence among MSM were conducted in STI clinics and anonymous testing sites. RITA assays were included as part of an unlinked anonymous surveillance programme using residues of specimens collected for syphilis serology among MSM attendees in a network of fifteen STI clinics for the past decade or so. In 1995–2001, no significant trend in HIV incidence was observed among these MSM; incidence ranged from a high of 3.29% in 1996 to a low of 1.46% in 1999 [32] [33]. The highest incidence was recorded among MSM aged 35–44 (4.2% in 1998 and 3.2% in 2001). HIV incidence was consistently higher in STI clinics outside London (median 0.97% per year) compared with those in London (median 3.0% per year). These estimates had wide confidence intervals, however, making the interpretation of the results difficult and underscoring the need for adequate sample sizes.

Incidence rates reported among MSM attending STI clinics in the US have been extremely high, particularly among those who report injecting drugs. Among attendees of an STI clinic in San Francisco, the annual HIV incidence estimate for MSM in 1989–98 was 6.6%, and 8.2% among those who also inject drugs. This compared with 7.5% among PWID and 0.36% among heterosexuals. Although HIV incidence fluctuated over the study period, there was no statistically significant trend over time [28]. In another study, HIV incidence amongst MSM attending an STI clinic in the same city was estimated at 4.9% (CI 2.3–8.3) in 1999, decreasing to 2.7% (CI 1.4–4.8) in 2006 (a non-significant trend) [34]. RITA testing among men with early syphilis using remnant samples from STI clinics in Atlanta, San Francisco and Los Angeles, resulted in annual estimates of HIV incidence of 9.5% (CI 3.9–15.1) among all men, increasing to 12.0% (CI 4.5–19.4) among MSM alone [35]. In a separate study among men with

early syphilis in Los Angeles County between 1999 and 2002, the annualised HIV incidence was estimated to be 17% (CI 12–22%/yr), increasing to 26% (CI 19–33%/yr) among MSM [26].

Several studies focusing on MSM attending anonymous HIV testing sites in the US have also shown high incidence rates although these have tended to fluctuate considerably across cities and years. In studies including testing sites across nine US cities during 1991–97, HIV incidence was estimated to be 0.8% per year (CI 0.6–1.1) overall. The average annual estimate was highest among MSM (7.1% (CI 4.8–10.3)), compared with 1.8% (CI 0.8–4.0%) among PWID and 0.5% (CI 0.4–0.7%) among heterosexuals [18][37]. In a similar study in 1997–2001, MSM were also the group with the highest proportion of recent infections (26%) and had the highest association with a recent infection (aOR of 1.57 (CI 1.2–2.3)) [19].

Lower incidence rates were reported among MSM attending anonymous HIV testing sites in San Francisco. The decrease from 4.1% in 1999 (CI 2.0–7.1) to 1.7% (CI 0.7–3.6) in 2003 was no statistically significant decrease over time [34]. Consistent findings were reported in a similar study [36]. It was noted that, despite a lack of significant increases in HIV incidence, there had been increases in both diagnosed STIs and self-reported risk behaviours among MSM in San Francisco. The estimated total number of new infections during 2006 was 975 (CI 801–1082), of which 851 (CI 732–1023) were among MSM [37]. In New York, for 2001, estimates of HIV incidence among MSM attending STI clinics were found to be 2.5% per year (CI 2.1–2.8) compared with 0.39% (CI 0.28–0.50) for all attendees [15].

Incidence studies at testing sites have also provided the opportunity to identify sub-groups at higher risk of HIV. For example, higher rates of HIV incidence rates were reported among MSM who report injecting drugs compared with those who do not (4.8% vs. 2.1%) [30] [38]. Higher estimates of HIV incidence were also reported among amphetamine users anonymously testing for HIV in San Francisco during 2001–02. Overall incidence estimates were comparable to previous studies, however men who reported amphetamine use had an estimated HIV incidence three times greater than non-users: 6.3% (CI 1.9–10.6) vs 2.1% (CI 1.3-2.9). HIV incidence was higher still among MSM who reported sex while under the influence of amphetamines (7.7%, CI 2.4-13.0) [39].

A number of publications have reported incidence rates among MSM based on RITA-tested samples from newly diagnosed individuals. In Ontario, Canada, for example, incidence in 1999 was estimated to be 2.5% among newly diagnosed MSM, and 6.5% among those who also reported injecting drug use [40]. In a larger study conducted on all newly diagnosed infections between 1998 and 2003, overall HIV incidence estimates were somewhat lower among all MSM (2.1%/yr) and those who reported injecting drug use (2.3%/yr) [41]. France was the first country to introduce a RITA assay as part of routine HIV surveillance at the national level. Findings of the first population-based incidence estimates showed that between 2003 and 2008, HIV incidence among MSM, who account for almost half of all newly diagnosed cases nationally, was estimated to be approximately 1% per year [42].

Other studies have presented data on recent infections as a proportion of all cases newly diagnosed, often as part of routine surveillance (Annex 4, Figure 5). These studies consistently report a higher proportion of recent infections among MSM compared with other groups. During a pilot study conducted between 2005 and 2007 in Berlin aimed at incorporating RITA testing into national surveillance, over half (54%; CI 45–64%) of HIV diagnoses among MSM were assigned as recent infections compared with 16% (CI 0–39%) among individuals with other risk factors [43]. Recent infections were significantly higher among MSM aged <30 (p=0.019). The pilot study offered the opportunity to explore the risk profile of MSM who were identified as recently infected [44]. While those infected were able to correctly recognise behaviours that presented a risk for HIV transmission, a degree of uncertainty, and optimism, were cited as reasons why men described engaging in risky sexual behaviour.

Recently acquired HIV infections have been monitored for over a decade (1996–2005) at the Brighton and Sussex University Hospital in the UK [45][16]. Data have shown an increasing proportion of individuals who were newly diagnosed with HIV and likely to have been recently infected. MSM accounted for the overall increase, reaching a level of over 50% in 2002, 2004 and 2005. In contrast, the proportion identified among heterosexuals remained stable and low [45].

A similar high proportion of recent infections among MSM have been seen through routine RITA testing among all newly diagnosed HIV infections in France, with 46% (41.0–44.6) of newly diagnosed infections likely to have been recently acquired between 2003–05, and 43% during 2003–06 [23] [25]. Compared with male heterosexuals, the odds of MSM being recently infected was estimated to be 1.85 (CI 1.59–2.15) [23]. Higher proportions of recent infections were seen among MSM in Catalonia, Spain, in 2003 and 2005, where MSM accounted for 62.5% of all recent infections [22].

Following a rise in the number of new infections diagnosed in Victoria, Australia, between 1999 and 2000 (increasing from 129 to 197), stored samples were RITA tested. Overall, 26% of cases in 1999 and 34% of cases in 2000 were likely to have been recently infected. These findings provided evidence of ongoing and possibly increased transmission of HIV in the state. Of all recently acquired infections, 77% were among MSM and 9% were among MSM who were also PWID [46].

RITA testing among newly diagnosed cases of HIV in the US during 2006 was used to estimate the total number of new infections during the same year in all 50 states and the District of Columbia. MSM accounted for 53% of all new infections, with an estimated 28 700 new infections (CI 24300–33000), compared with 16 800 (CI 14200–19400) among heterosexuals and 6 600 (CI 5300–7900) among PWID, representing 31% and 12% of all new infections, respectively [18] [21].

Local RITA testing data has also been used to estimate the number of all new HIV infections in several US cities. In San Francisco, the estimated total number of new infections in 2006 was 975 (CI 801–1082) and includes 851 (CI 732–1023) new infections among MSM [37]. Population-based HIV incidence estimates among MSM in Florida during 2006 indicated that overall there were 656.1 new infections per 100 000 MSM in 2006, including an estimated HIV incidence of 2154.2/100 000 among black MSM, 764.4/100 000 among Hispanic MSM and 392.6 among white MSM [47].

2.4 Recently acquired HIV infections in people who inject drugs

Studies using RITA tests to measure HIV incidence among people usually focus on MSM: there are more studies of this type on MSM than PWID, especially in Western Europe, largely because the HIV prevalence is generally much lower among PWID; also, PWID constitute only a small proportion of all new diagnoses. Consequently, very large sample sizes are required to adequately estimate HIV incidence in this risk group. Where studies in drug-using populations have been conducted, data have often been pooled over many years to obtain a more robust estimate.

RITA testing among PWID was conducted in a number of settings in the US (Annex 4, Figure 6). Between 1996 and 1998, among PWID attending anonymous HIV testing centres in San Francisco, no recent infections were reported among those who described themselves as heterosexual; however, 13 out of 58 men who also reported sex with other men had a recent infection, resulting in an incidence estimate of 6.3% (CI 2.6–13.8) in this group [16]. Similarly, no recently acquired HIV infections were identified among clients of a drug treatment clinic in the same city (1070 samples were collected between 1995 and 1998) [48]. PWID attending municipal STI clinics in San Francisco were also RITA tested during the ten-year period between 1989 and 1998 and accounted for less than one in twenty new diagnoses. The annual HIV incidence was estimated at 7.5% in all PWID, and 8.2% among MSM [28]. Despite some differences over the years, observed trends were not robust due to a small number of recent infections among this population. During 1990–2002, 3600 PWID attending a drug detoxification programme in New York City were RITA tested. A significant decline in HIV incidence was observed, with incidence falling from an average of 3.1% per year between 1990–1995, to 0.9% per year in 1996–2002 ($p<0.05$) [49][31]. A large scale study involving retrospective RITA testing of sample collected through population-based HIV surveillance in New York during 2001 provided an incidence 0.8% per year (CI 0.6–0.9) among PWID [15].

Over a three-month period in 1999, HIV incidence among newly diagnosed individuals in Ontario, Canada, was estimated to be 0.78%/yr among PWID, compared with 2.5%/yr among MSM, and 6.5%/yr among MSM who also injected drugs [40]. In a larger study conducted between 1998 and 2003 in the same province, overall HIV incidence estimates were lower: 0.22%/yr in PWID, 2.1%/yr among MSM, and 2.3%/yr among MSM who also reported injection drug use [41].

Several studies have reported the proportions of newly acquired infections among newly diagnosed individuals based on the results of RITA tests (Annex 4, Figure 7). In a network of STI clinics across nine US cities in 1991–97, 5% of PWID tested were reported as recently acquired, resulting in an incidence estimate of 1.8%/yr [18]. A much higher proportion (21%) was identified in a similar study across ten US sites which was conducted from 1997 to 2001 [19], while national estimates for the whole of the US suggested that approximately 12% of PWID diagnosed with HIV acquired their infection recently, equivalent to an estimated 6600 new infections per year [21]. In a three-year cross-sectional study of hospital outpatient clinics, STI clinics and community-based VCT sites in Catalonia, PWID accounted for 14.4% of all recent infections, increasing to 42.9% of recent infections diagnosed in community-based VCT sites, although the sample sizes were small [22].

As part of routine national HIV surveillance in France, data on recent HIV infections were reported for the years 2003 to 2006. Fifteen percent (CI 10.0–19.2) of PWID were diagnosed with recent infection. It should be noted, however, that this group accounted for less than 1% of all recently acquired infections [23]. Population-based modelling of HIV incidence using national data showed that of the 6940 (CI 6200–7690) estimated persons diagnosed with HIV infection in France in 2008, 70 were among IDU (CI 0–190), with an estimated incidence of 0.09% [42]. Retrospective testing of 459/1027 HIV-infected blood donors in France in 1992 and 2006 indicated that 18% (2/11) of identified HIV infections among PWID were likely to have been recently acquired (risk factor information obtained through follow-up of HIV-positive donors) [24].

2.5 Recently acquired HIV infections in migrants and ethnic minorities

Migrant populations across Europe and North America are comprised of substantially different subgroups, some of which are well established and have been resident, or remain in their host country for many years, while others are highly mobile. Quantifying recent infections among this group is therefore extremely challenging and is likely to vary both over time, and between geographical locations. Furthermore, the viral subtype varies between groups of different geographic migrant origin and is known to affect the RITA assay's ability to detect the differences in antibody binding which serves as the basis for distinguishing recent infections among new diagnoses [50] [51].

In a three year cross-sectional study of HIV testing sites in Catalonia (Annex 4, Figure 9), immigrants accounted for 27% of all recent infections, increasing to 79% of recent infections when diagnosed in community-based VCT sites [22]. When stratified by world region of origin, the highest proportion of recent infections was seen among those originating in South and Central America: over half of all infections were recently acquired (55%), compared with 16% people from Eastern and Central Europe, and 8.8% among sub-Saharan Africans.

In RITA testing among all new diagnoses in France between 2003 and 2006, the highest proportion of recent infections was seen among French nationals, where 35% of new diagnoses were likely to have been recently acquired, compared with 24% among other European nationals, 19% among North Africans, 8.4% among sub-Saharan Africans, and 21% among those of other or unknown ethnicity [23]. A multivariate analysis of independent factors showed a lower likelihood of recent infections among sub-Saharan Africans (aOR 0.31) and other non-nationals (aOR 0.65) when compared with French nationals (aOR 1) [25]. In 2008, HIV incidence among non-nationals in France was significantly higher than among French nationals. The annual HIV incidence was 0.05% (CI 0.04–0.07) among foreign-born women and 0.04% (0.02–0.05) among foreign-born men, compared with 0.04 (0.03–0.05) and 0.06 (0.04–0.08) in French-born women and men, respectively [42].

Among blood donors in France in 1992–2006, the proportion of HIV-positive donations from individuals with a recently acquired infection has remained largely stable (between 21% and 26%) [24]. Overall, approximately one in five HIV-positive donations among French nationals or other European nationals were likely to have been recently acquired, compared with 15% and 7.7% among North Africans and sub-Saharan Africans, respectively.

During a three-year survey (2004–07) in clinical centres that offered primary healthcare to illegal immigrants in Italy, HIV prevalence was 0.97% (almost double the national HIV prevalence) [52]. RITA testing indicated that six persons (22.2%) were likely to have been infected in Italy, with a further four having acquired their infection prior to migration. Recently infected individuals were migrants from sub-Saharan Africa (three), Eastern Europe (two) and Latin America (one).

HIV incidence among attendees of HIV treatment/testing sites and STI testing sites in several US cities varies by ethnicity (Annex 4, Figure 8). In three studies among individuals attending anonymous HIV testing sites in San Francisco between 1996–1998 and 1996–2002, overall annual HIV incidence estimates ranged from 1.9–2.5% among those of white ethnicity, 0.8–1.1% among African Americans, 1.1%–3.1% among Hispanics and 0.6–2.0% in those of other ethnicity [30] [16] [28]. HIV incidence among African Americans during this time frame showed a significant decline in incidence from 1.5% in 1989 to 0.68% in 1998 (OR 0.84; CI 0.72–0.97) [28]. These estimates were much higher than those reported among individuals attending publically funded testing sites in New York City where the annual HIV incidence rate among white persons was 0.38%, compared with 0.43% among Blacks, 0.22% among Hispanics, and 0.11% in Asian Pacific Islanders and Native Americans [15]. Among men with early syphilis in Los Angeles, recent HIV infections accounted for 17% of new diagnoses among African Americans, 33% among Whites, 42% among Hispanics, and 8.3% among other ethnic groups [26].

In a cross-sectional survey among young Asian and Pacific Islander MSM in 2000–01, recruited though venue-based time and space sampling in San Francisco, HIV incidence was estimated to be 1.8%/yr [53], similar to the overall incidence estimate of 2.0%/yr reported among Asians and Pacific Islanders for the period 1996–2002 [16] [53] and to the 2.2% reported among all MSM in San Francisco.

In ten US cities between 1997–2001 (Annex 4, Figure 8), recent HIV infections affected African Americans (16% of all recent infections), Hispanics (16%), Whites (29%), Asians (38%) and other ethnic groups (23%) [53] [19]. In nine US cities between 1991–97, recent infections affected Blacks (59% of all recent infections), Hispanics (19%) and Whites (21%) [18]. National estimates of HIV incidence by ethnicity indicate that while individuals of white ethnicity account for 35% of all recently acquired infections, the majority of new infections in the US are among those of black ethnicity, accounting for 45% of all infections; 17% of new infections are among Hispanics. Expressed as a rate per 100 000 people, infections among Whites were substantially less than in Blacks or Hispanics (11.5 compared with 83.7 and 29.3, respectively) [20] [21]. In the absence of more data on the relative proportion of long-standing infections among immigrants, data such as these are difficult to interpret. These data could indicate a preference for, or better access to, non-official HIV testing sites among this population, or a higher HIV incidence among this group.

2.6 Summary

The development of RITA assays has provided a practical alternative to lengthy, expensive, and logistically challenging cohort studies to estimate HIV incidence in most at-risk populations.

Setting and target population in the reviewed studies varies from country to country and often reflects the local epidemiology as well as resource and time constraints. In total, 48 papers, three abstracts and one poster describing HIV incidence studies using RITA assays were identified in the review, with the first study published in 1998. Most of the studies were conducted in the United States with detuned and BED assays and primarily focusing on the incidence of HIV among MSM. The United Kingdom is among the countries with the longest history of using RITA assays, most notably among MSM through UAT (unlinked anonymous testing) HIV sentinel surveillance. Germany and France are among the countries that have implemented RITA testing as part of the national or regional surveillance; in Spain, the Netherlands and Italy, RITA testing has been used in sentinel surveillance studies or in specific (cohort) studies.

Early publications focused on key groups such as pregnant women, young people, IDU and MSM, or settings such as STI clinics and anonymous HIV testing sites, which put greater emphasis on the use of RITA as part of the surveillance of newly diagnosed individuals – notably in France, but also in the UK, Italy and Germany. Technological developments, such as the use of dried blood spots for collecting HIV samples and automated RITA assays, have facilitated the collection of these data.

Published HIV incidence estimates varied widely. Estimates among young people and migrants were generally well below 1% per year in most settings, except among STI clinic attendees, where estimates reached 4%. The proportion of newly diagnosed HIV infections that were likely to have been recently acquired showed greater variability, ranging from 0 to approximately 60%, with substantial differences between different geographic locations and ethnic groups.

Estimated HIV incidence among MSM was, not surprisingly, much higher but generally below 5%/year, with the exception of MSM with concurrent STIs (in particular early syphilis and gonorrhoea) and users of recreational drugs (e.g. amphetamines). Estimates obtained through anonymous testing sites were often as high as through STI clinics. Where proportions of recently acquired infections were presented, the majority of estimates for MSM were at around 50%.

There are noticeably fewer studies conducted among people who inject drugs, possibly reflecting the fact that the majority of RITA publications were from Western Europe and the US, where PWID represent a smaller proportion of all newly diagnosed HIV infections. There was a wide variability in incidence estimates, ranging from 0% to 8% per year. Where only proportions were reported, recent infections represented between approximately 6 and 15% of newly diagnosed infections.

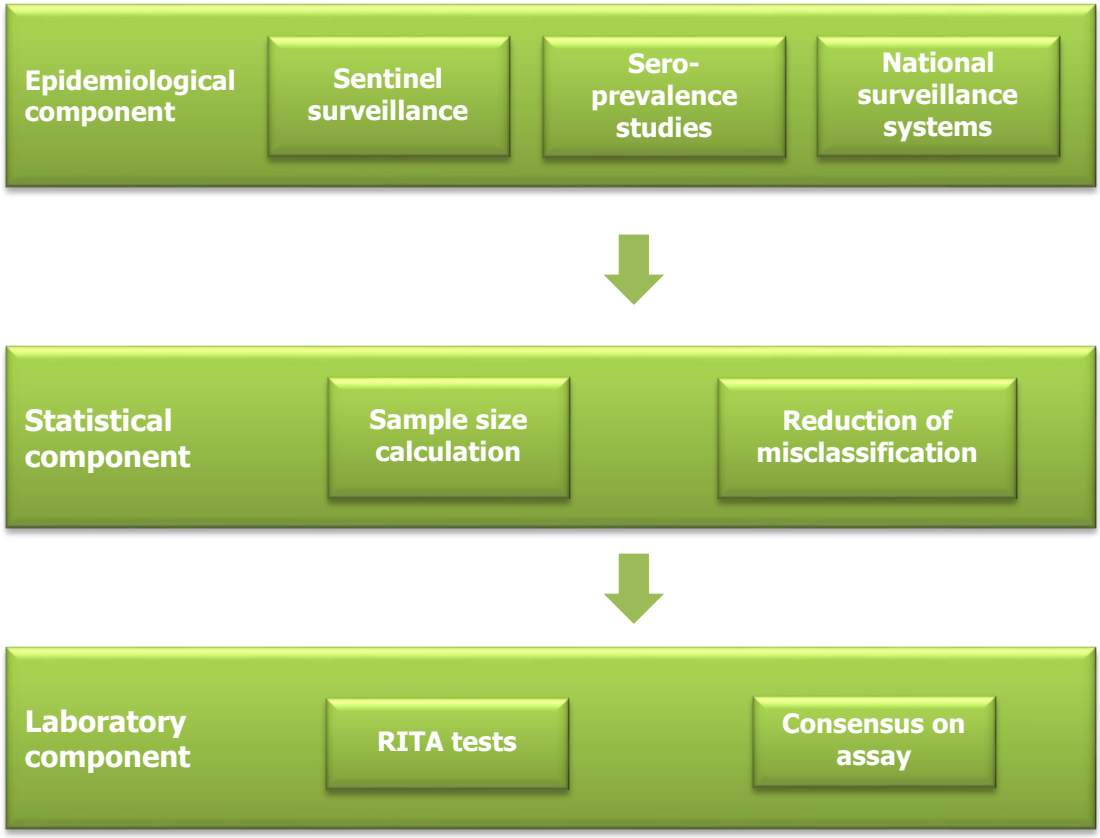
3 Framework for monitoring recently acquired HIV infections

3.1 Epidemiological considerations

The purpose of the framework is to guide the use of laboratory assays for recent HIV infection in combination with additional information from surveillance systems or studies as a basis for the estimation of incidence in key populations in Europe (Figure 1). The framework takes also into account, and draws from current recommendations from the WHO Technical Working Group on HIV Incidence Assays [10]. Prevention efforts should be informed by the public health utility of monitoring and reporting the proportion of all newly diagnosed infections that are likely to have been recently acquired at a national and regional level.

Laboratory tests to identify recently acquired HIV infections require only a single sample of diagnostic sera, collected from an individual at a single point in time. This offers a practical alternative to lengthy, expensive, and logistically challenging cohort studies. As a result, laboratory tests for recent HIV infection have been applied across a variety of settings, using a range of epidemiological approaches. Study settings and target populations often vary by country and reflect the local epidemiology as well as resource and time constraints.

Figure 1: Framework for HIV incidence



3.2 Sentinel surveillance

The literature review indicated that RITA assays were most frequently implemented through convenience sampling as part of sentinel surveillance programmes [16] [17] [22] [28] [29] [30] [31] [32] [54] [55]. These programmes aimed to collect high-quality, consistent and detailed data from a limited number of sites. Sentinel surveillance programmes generally targeted services and settings that are used by individuals who are either of specific interest (people at a higher risk of infection) or considered to be representative of a larger population (people attending sexual health clinics or needle exchange centres). In general, authors were cautious about the generalisability of their findings to individuals who were tested in alternative settings. However, HIV incidence estimates among risk groups attending settings such as STI clinics were largely considered to be reflective of individuals who attend similar HIV testing services and have the same risk for HIV infection.

The UNAIDS/WHO working group on global HIV/AIDS and STI surveillance has produced a document titled 'Guidelines for conducting HIV sentinel serosurveys among pregnant women and other groups' [56] which provides information on setting up sentinel HIV surveillance studies. This type of study provides a statistical method of estimating HIV incidence (known number of HIV-negative, HIV-positive and recently infected individuals). Details of the statistical methods described by Janssen seroprevalence are available in a publication of the WHO Technical Working Group on HIV incidence assays [6] [10].

Advantages and disadvantages

Obtaining data through sentinel surveillance systems is generally less expensive than the active surveillance of a total population; sentinel surveillance also yields higher quality data than passive systems. This is largely because it is logistically easier to obtain higher quality information from a smaller population. However, it can be difficult to ensure the representativeness of the sample, particularly if recruitment is in a clinical setting where there may be an over-representation of people seeking care due to symptoms and hence an upward bias in the incidence estimate. Also, individuals may be motivated to get tested as a result of recent high-risk behaviour.

Example

In the UK, an estimated 95% of all HIV infections amongst MSM are diagnosed in STI clinics. An unlinked anonymous HIV prevalence serosurvey using routine syphilis serology specimens from MSM who attend 15 STI clinics (representative of the approximately 200 clinics in the UK) was established in 1990 [33]. Between 1995 and 2001, 3565 of 43 100 MSM attendees were confirmed to be HIV-1 positive. Based on RITA assays, the overall annual HIV incidence in this group was estimated to be 1.46% (95% CI 0.77–2.66) in 1999 and 3.29% (95% CI 2.06–5.23) in 1996, with a median of 2.4%, and no overall significant trend.

3.3 Seroprevalence studies

In cross-sectional or seroprevalence studies, data are collected for the entire study population at a single point in time to examine the relationship between HIV and recent HIV infection. Also collected are other variables of interest such as risk group, age, sex, etc. If generalisations from the findings should have any validity, cross-sectional studies should be representative of the entire population and include hard-to-reach groups and marginalised groups whenever possible. As it is most often impractical to survey the entire population of interest, a sampling strategy is used instead. Random samples are ideal: individuals are chosen at random from the population of interest, with all individuals having an equal chance of being included. Findings are assumed to be largely representative of the population as a whole, but are difficult to implement (especially for hard-to-reach populations). Convenience sampling is a non-probability sampling technique: individuals are included in the study based on their accessibility; this may include surveys in bars, clubs, needle exchange programmes, etc. Studies of this type are more common, but not considered to provide a representative sample of the whole population. They are however, quicker, less expensive, and easier to perform.

RITA assays are included as a component of a number of seroprevalence studies targeting populations such as MSM, blood donors, ethnic minority MSM, pregnant women, and migrant populations [18] [24] [54] [28] [29] [48] [49] [52] [53] [57] [58]. These studies were designed to assess the prevalence of HIV within a given population and identify those infections likely to have been recently acquired, i.e. within a well-defined time-frame, typically the previous four to six months.

One seroprevalence survey used a quota sampling approach, whereby consecutive non-duplicate samples were selected until a predefined number of samples was obtained for each risk group (MSM, heterosexual men, heterosexual women) [28]. These studies highlight the need for a large sample size of persons tested for HIV in order to obtain accurate estimates of incidence and to compare findings over time and by characteristics of interest.

In cross-sectional or prevalence studies, in which the number of HIV-negative, positive and recently infected individuals is known, HIV incidence can be calculated using the approach described by Janssen et al. and described in a publication by the WHO Technical Working Group on HIV incidence assays [6] [10]. These studies highlight the need for a very large sample size of persons tested for HIV in order to obtain accurate estimates of incidence and to compare findings over time and by characteristics of interest.

Advantages and disadvantages

Estimating incidence of HIV from a single cross-sectional survey has several advantages for surveillance over the conventional method of measuring incidence using longitudinal cohort studies. Cross-sectional studies are generally relatively quick and easy to conduct, with no long periods of follow-up, and are primarily used to assess disease prevalence in a specified population and for the allocation of health resources. In this type of study, data on all individuals are collected in one go and make it possible to simultaneously investigate multiple outcomes and exposures.

There are also disadvantages. Non-response is a particular problem affecting cross-sectional studies and can result in bias, which is a particularly tricky problem when the characteristics of non-responders differ from those of the responders. With regard to RITA testing, there is the additional problem that participants may not wish to provide a biological sample or the provided samples are inadequate. Furthermore, if information about risk factors is collected retrospectively, there is the additional risk of recall bias. Cross-sectional studies have to be sufficiently large in order to detect the occurrence of a relatively uncommon event (such as recent HIV infection) at a significant statistical level. Trends from repeated cross-sectional studies are often difficult to compare due to differences in the population and often result in large amount of uncertainty around the point estimate.

Examples

In San Francisco, a cross-sectional analysis of MSM presenting for HIV testing at municipal STI clinics (2000–04) and at anonymous testing sites (ATS) for HIV (2000–03) assessed the concordance between two RITA tests (Vironostika-LS and BED-CEIA) and examined trends in HIV incidence by looking at repeat testing data [31]. In total, 14 956 samples were collected (STI=9182, ATS=5828) and 658 (4.40%) were confirmed HIV-positive (STI=439, 4.78%; ATS=219, 3.76%). These results provided clear evidence of ongoing transmission among MSM attending STI and ATS testing sites, with the temporal trends of all three HIV incidence estimation methods showing broadly similar trends, ranging from 3.28–3.52% in 2000 to 4.11–4.01% in 2004 (among STI clinic attendees), and between 1.90 and 4.00 in 2000 to 1.78–2.26 in 2003 (ATS attendees). For all years (except 2001 in STI clinic attendees), incidence estimates among repeat testers was lower than RITA-derived estimates.

In a three-year cross-sectional study in Spain, carried out between 2003 and 2005, confirmed HIV-positive samples from adult patients without a previous confirmed HIV diagnosis were combined were RITA tested (Vironistika-LS) [22]. Samples were collected through a network of 28 laboratories, serving a network of STI clinics, community VCT sites, primary health centres and hospital outpatient clinics. In total, 478 932 HIV tests were performed, and 5 800 (12.11%) were confirmed HIV-positive; 3 444 individuals were RITA tested, and 660 (19.2%) were found to be recently infected. Recent infections were more common in men (79.8%), Spanish nationals (73.5%), those aged 30–40 years (44.0%), and MSM (62.5%). Higher proportions of recent infections were also seen among attendees of STI clinics and primary health centres, where 21.5% (N=276; 95% CI 19.2–23.7) and 23.4% (N=82; 95% CI 18.9–27.8) of all individuals were recently infected.

3.4 National surveillance system

In order to address the selection bias which is inherent in other epidemiological approaches and compensate for the limited potential to extrapolate findings to the wider HIV testing population, a number of regions and countries have applied RITA to all newly diagnosed infections at the local, regional and national level [7] [15] [20] [21] [23] [37] [42] [43] [44] [46] [59] [60]. Studies have been largely confined to countries with an established system for the unlinked anonymous surveillance of HIV, e.g. France, Germany, and Austria.

Advantages and disadvantages

Implementing RITA as part of an existing national surveillance system will provide a more comprehensive picture of current trends in HIV transmission. There are however, a number of limitations when using case-based surveillance systems. Differences in access to HIV diagnostic services may result in a bias in reported HIV diagnoses as local and national campaigns promote testing. Furthermore, individuals motivated to get tested as a result of recent high-risk behaviour or due to symptoms associated with primary HIV infection are more likely to get tested, which will result in inflated population-level HIV incidence estimates. The completeness of an HIV surveillance system may be adversely affected by reporting delays. Stratifying HIV incidence estimated by risk group requires population estimates for those groups, which can be difficult and introduce further statistical complexity and uncertainty.

Example

In France, RITA has been a routine component of the national surveillance of HIV since 2003. All new HIV diagnoses are reported to the Institut de veille sanitaire (InVS) and accompanied by a dried serum spot for RITA testing. Between 2003 and 2006, 14 155 new HIV diagnoses were reported [23]. Of these, 10 855 came with samples for RITA testing, of which 511 (23.1%; 95% CI 22.3–23.9%) were identified as recent HIV infection. Recent infections were higher among MSM (42.8%) than heterosexuals (16.3%) and among French nationals (27.0%) compared with Africans (8.4%). In a multivariate analysis, the risk of recent infection (RI) was greater for MSM (aOR=1.8), those of French nationality (aOR=3.9), those of a high socio-economic status (aOR=1.2), those tested for HIV after a risk exposure (aOR=1.4) and those who had undergone three or more tests during their lifetime (aOR=2.5). However, the risk of RI decreased with age. The proportion of RI was stable between 2003 and 2006, with the year of diagnosis not significantly associated with RI. The authors also reported no significant changes to HIV screening policies or the rate of HIV screening during this timeframe.

3.5 Cohort studies

Historically, the gold standard for estimating HIV incidence used to be prospective cohort studies of individuals at risk for HIV infection who are followed over time with serial blood specimen collections. Cohorts comprise a group of individuals who share a common behaviour or risk for HIV infection such as MSM or commercial sex workers (CSW), or are users of specific services such as sexual health clinics. Applying RITA as part of a cohort study allows for a direct comparison between observed HIV incidence and that measured through the application of RITA. It is also possible to include specific behaviour and exposure information in the study. In cohort studies, where the number of HIV-negative, positive and recently infected individuals is known, HIV incidence can be calculated using the approach described by Janssen et al. and described by the WHO Technical Working Group on HIV incidence assays [6] [10]. In situations in which samples are unlinked and anonymised prior to testing, an additional correction for repeat attendance during the study period can also be included.

Advantages and disadvantages

Cohort studies have the advantage of allowing the simultaneous measurement of multiple outcomes/exposures and can be used to directly measure incidence and prevalence. However, generalising these findings to the wider populations from which they were drawn is dependent on the degree to which they are comparable. Cohort studies are subject to important sources of error which can bias these estimates, for example there may be a significant selection bias among those who agree to participate in cohort studies. These individuals may have risk behaviour which differs from other members of the population. Repeated follow-up of cohort participants and counselling sessions can also impact risk behaviour. Also, individuals may be lost to follow-up as a result of death, migration, or refusal to continue participating. Losses to follow-up may be related to the exposure, outcome, or both, and if correlated with either exposure or outcome, can lead to serious bias. In addition, cohorts are logistically difficult to implement, expensive, often not timely, and prone to biases that can distort estimates of HIV incidence for the underlying populations.

Example

One study directly compared HIV incidence estimates from RITA and observed HIV incidence in the cohort. This study used the AIDSVAX Phase 3 clinical trial cohort of HIV-negative MSM and women aged 18 to 60 years, who were at risk for HIV-1 infection through sexual activity. Individuals were recruited between 1998–99 across 61 sites in the US and the Netherlands. The RITA-based HIV incidence estimate of 2.91%/yr (CI 2.30–3.53%/yr) compared well to an observed incidence of 3.10% (CI 2.57–3.63) [12]. These findings suggest that RITA tests provide a cost-efficient and timely method of estimating incidence compared with a cohort study design although these estimates may vary, particularly if the number of new infections in the population is small.

3.6 Statistical considerations

Sample size calculations

Sample size calculations to determine the number of individuals that should be included in a study are an essential component of any study design, particularly when the study is aimed at generating robust estimates of HIV incidence with a predefined level of certainty. Estimates derived from larger studies are less likely to be due to chance alone, but may have significant implications in terms of costs. Appropriate sample size calculations will result in narrower confidence intervals, more reliable incidence estimates, and an enhanced ability to detect significant changes in incidence over time. Data required for a sample size calculation is detailed in the WHO guidance for the use of RITA assays [10]. In brief, data should include:

- the estimated prevalence and incidence of HIV in the population to be studied;
- the mean window period of the RITA assay and its coefficient of variation; and
- the false-recent rate (FRR) and its coefficient of variation.

These data can be used to estimate the sample size using the 'sample size worksheet' as described in the WHO guidance (available from http://www0.sun.ac.za/sacema/collaboration/ABIE/) [10]. In cohort studies, sample size calculations should also take into account the expected response rate and estimated losses to follow-up. The impact of sample size is illustrated in Figures 1 to 5 in Annex 5, with an estimated annual HIV incidence of between 1% and 5% and an increasing population size from 100 to 50 000.

The literature review highlights considerable variation in the statistical methods to measure incidence, and few studies provided robust estimates of incidence. Where available, calculations of confidence interval were based on different underlying assumptions (binomial, Poisson, or normal distributions) as well as using adjustment procedures (for example Bonferroni adjustment). Furthermore, some studies did not report confidence intervals, which made interpretation difficult.

Annual incidence rates of HIV are likely to be well below 1–2% in most populations (and below 4% in high-risk settings such as STI clinics), and large sample sizes are required to confidently estimate incidence and monitor trends over time. Many of the studies identified very few cases as having recently acquired their infection and the final estimates were presented with very wide confidence intervals. Multiple years were often combined to increase the number of included individuals and provide more robust estimates of HIV incidence, but this limited their public health utility in monitoring time trends.

Direct approaches addressing the potential misclassification of a RITA minimises, but does not completely eliminate the FRR. In settings where there is complete and accurate patient level information, the residual FRR – after direct FRR minimisation has been applied – is likely to have a negligible effect on the sample size and may therefore be omitted.

Minimise misclassification of long-standing infections as recent

A proportion of individuals with advanced HIV infection, AIDS, or receiving antiretroviral therapy maybe misclassified as having recent HIV infection due to the use of the existing assays. The false recent rate (FRR) is therefore the fraction of non-recent infections that are incorrectly classified as 'recent' as a result of applying a RITA. There are two main approaches to tackling FRR, either directly, through reclassification of false recent infection as long-standing on a case-by-case basis, or indirectly through including an estimated overall FRR correction when estimating HIV incidence. If a RITA includes two or more RITA assays, the agreement between these assays may represent a viable alternative.

Direct approach

The case-based or direct approach uses information collected from the patient to exclude falsely classified recent cases and therefore minimises, but not eliminates, the FRR. The following case-based criteria can be used to exclude specimens classified as 'recent' by the assay:

- CD4 count of <200 cells/mm^3, within four months of the sample date.
- AIDS-defining illness reported within a year of the sample date.
- Previous HIV-positive test result that predates the sample date by more than the mean RITA duration.
- Laboratory-confirmed presence of ARV prior to the sample date.

Although a direct approach does not require a modified HIV incidence estimate, it is important to note that a proportion of infections may still be incorrectly classified if clinical/epidemiological and laboratory information is unavailable or incomplete and thus result in inflated estimates of HIV incidence in the study population.

Indirect approach

Panels of well-characterised specimens with long-standing infections can be used to estimate the FRR. Data can be drawn from a population corresponding to the populations for which incidence will be estimated. This estimate can then be used to adjust HIV incidence estimates.

For example, the annual incidence rate (I_r) can be estimated by:

$$I_r = \frac{R - \varepsilon P}{(1-\varepsilon)\omega N}$$

where the survey counts (N , P , R) are specified as follows:

N is the number of HIV-negative people in the survey

P is the number of HIV-positive people in the survey, and

R is the number of people classified RITA-positive,

and the calibration parameters are specified as follows:

ω is the mean RITA duration specified in units of years, and

ε is the false recent rate of the RITA.

For more details, please see the guidance produced by the World Health Organization Technical Working Group on HIV incidence assays [10].

Additional statistical approaches to adjust HIV incidence estimates

Two alternative methods have been proposed to deal with the misclassification of recent HIV infection as long-standing (false negatives) and long-standing infections as recent (false positives), namely the McDougal and Hargrove adjustments [12] [61]. Both use sensitivity and specificity corrections to adjust the window period and therefore redefine the number of infections defined as recent. A full discussion of these methodologies and their relative merits has been published elsewhere [13] [14]. Both methods underscore the need for better integration of epidemiological data to identify and exclude long-standing infections and the need for more robust, assay-specific window period estimates.

Minimise misclassification of recent infections as long-standing

Estimating false long-standing infections requires a similar approach to that described in conjunction with FRR estimates. However, minimising a false-long standing rate by using clinical, epidemiological and laboratory data is possibly be more difficult, and may include:

- previously documented HIV-negative test results within 180 days of the sample date;
- previously documented HIV RNA+/Ab- or p24 Ag+/Ab- within 180 days of sample date; and
- clinical symptoms of seroconversion.

Consensus of two tests of recent infection

Although these sources of error may be more challenging to estimate, and are rarely addressed in the published literature, they are nevertheless important to consider.

3.7 Estimating HIV incidence

HIV incidence from cross-sectional data

One of the most frequently used approaches to estimate HIV incidence in the published literature was described by Janssen et al. [6] in their initial (1998) publication:

$$I=(n_{dt}/N_d)(356/T)(100)$$

where n_{dt} is the number of recently acquired infections, N_d is the size of the susceptible population (HIV-negative, plus recently infected), and T is the size of the window period in days.

Confidence intervals for the HIV incidence estimates were most frequently calculated using a Bonferroni procedure, reflecting sampling variability and uncertainty in mean time between seroconversions. The Bonferroni 95% CI for HIV incidence is:

$L_{95} = (n1_{dt}/N)(365/T_1)*100$, where $n1_{dt}$ is the *lower* 97.5% CI from the Poisson assumption, N is the number of HIV-1 negative persons plus the number with RITA-reactive and non-reactive test results, and T_1 is the *upper* 97.5% CI for mean time between seroconversions.

$U_{95} = (n2_{dt}/N)(365/T_2)*100$, where $n2_{dt}$ is the *upper* 97.5% CI from the Poisson assumption, N is the number of HIV-1 negative persons plus the number with RITA-reactive and non-reactive test results, and T1 is the *lower* 97.5% CI for mean time between seroconversions.

Additional approaches for estimating HIV incidence as a rate, incorporating a correction factor for the FRR, and CI estimation assuming a Gaussian/normal distribution can be found in the WHO guidance for estimating HIV incidence [62].

3.8 HIV incidence from case-reporting systems

While it seems especially promising to use recent HIV infection testing among reported HIV diagnoses at national level, there are several specific difficulties with regard to deriving a valid incidence measurement. Unlike cross-sectional surveys, a case-reporting system collects information only for individuals with positive test results and generally cannot provide information on those who were negative. Therefore, the denominator of the cross-sectional formula, i.e. the number of people at risk for HIV infection, is not available. Furthermore, HIV-infected individuals are diagnosed with a time delay that is variable. Another approach is needed to derive an incidence that can be generalised for the population targeted by the surveillance, and to take account of the fact that negative test results are not reported.

Such an approach has been first described by Lee et al. for the estimation of the national HIV incidence in the USA [63]. The method was applied by Hall et al. (2008) to provide national incidence estimates for 2006 [21]. The statistical framework is described in detail by Karon et al. [64]. This approach represents a good opportunity to

improve large-scale surveillance of HIV dynamics, especially where a framework of HIV case reporting already exists and can provide data on testing patterns. It has been adapted in France, where testing for recent HIV infection has been routinely performed since the beginning of HIV case surveillance in 2003 [42].

Providing that the window period (or RITA duration) of the test for recent HIV infection is defined and that information on HIV testing history is collected for new diagnoses tested for recent HIV infection, the principle of the model developed by Karon and colleagues can be described as follows:

HIV diagnoses are first stratified into subpopulation groups according to demographic variables to constitute homogeneous risk groups. The model considers the diagnoses observed as recent HIV infection as a random sample drawn from the population of HIV infections occurring within one year (incident cases). The corresponding sampling probability is calculated as the probability of being tested within one year following infection (p_1) times that of being detected as recently infected while diagnosed within a year after infection (p_w). The first probability p_1 is estimated separately among individuals who report a negative test before diagnosis (termed repeat testers) and those assumed to be diagnosed at their first test (new testers). These conditional probabilities are primarily determined by testing history (p_1 for repeat testers), the proportion of HIV infection diagnosed at AIDS stage (p_1 for new testers) and the mean RITA duration (for p_w). The number of incident cases within a subpopulation is then derived from the size of the sample divided by its sampling probability.

There are several limitations issuing from the chosen model and the available data. The reliability of incidence estimates is primarily dependant on how well the history of previous tests reflects actual testing patterns and how the window period defined for the test can be applied to the general population [42] [64]. This approach represents a good opportunity to improve large-scale surveillance of HIV dynamics, especially where a framework of HIV case reporting (a) already exists, (b) can include testing for recent HIV infection, and (c) can provide data on testing patterns.

Study designs presented here are primarily aimed at countries with localised epidemics. Other strategies such as household surveys may be appropriate in countries with a generalised epidemic where the HIV prevalence in the adult population exceeds 1%. Detailed guidance on household survey methodologies can be found in the 'Guidelines for measuring national HIV prevalence in population-based surveys', produced by the UNAIDS/WHO working group on global HIV/AIDS and STI surveillance [10].

3.9 Laboratory considerations

The absence of reliable assays for the estimation of HIV incidence represents a significant public health gap. Recognising this need to drive the identification and validation of novel, improved HIV incidence assays by engaging a broad range of stakeholders in a collaborative, focused partnership, the World Health Organization has convened a Technical Working Group on HIV incidence, comprised of epidemiologists, laboratory specialists, and public health officials. The group has worked to standardise terminology in the areas of assay calibration and validation, conducted a comprehensive literature review of studies on the assessment of assays, developed a standardised protocol for assay validation, and defined the specimen sample sets required for assay calibration and validation. The WHO Technical Working Group on HIV incidence assays also developed a framework for advancing assay development, evaluation, validation, and comparison [10]. More recently, the Consortium for the Evaluation of the Performance of HIV Incidence Assays (CEPHIA) was established as an international project funded by the Bill & Melinda Gates Foundation to evaluate the performance of HIV incidence assays. In 2013, a range of assays will be evaluated using well-characterised panels of HIV-positive samples. The CEPHIA group will be developing protocols for these assays to ensure standardised use and reproducibility of results when applied in different laboratories across the globe.

3.10 Considerations for implementation

Estimates of HIV incidence in key populations across the EU/EEA can bring a deeper understanding of the transmission of HIV and will allow better monitoring and evaluation of key prevention interventions. The use of RITA offers a unique opportunity to estimate HIV incidence at relatively low cost and in a timely manner. However, to maximise the public health benefits across Europe, it is critical that studies are coordinated using standardised and comparable laboratory, epidemiological and statistical methods. The outcomes of the CEPHIA project will be particularly important in informing the choice and comparability of RITA tests used in the European context.

The public health utility of tests for recent infection with HIV in Europe will largely depend on their capacity to generate robust HIV estimates and determine the proportion of recent infections in key populations which can be monitored over time. Appropriate sample size calculations during the planning phase of a study which uses RITA tests as well as correcting for biases will be essential. The effect of sample size on the confidence intervals for HIV incidence estimates of between 1 and 5%/year are illustrated in series of graphs in Annex 5. In general, published studies that have generated the most robust estimates of incidence have been those that have implemented RITA as a part of very large cohort studies or national surveillance systems.

While estimating HIV incidence in key populations remains the overall long-term goal of employing RITA testing, monitoring the proportion of recent HIV infections among all new HIV diagnoses is likely to be more feasible in the first instance. Trends in the proportion of recent HIV infections can provide a timely insight into transmission dynamics of key most-at-risk populations, thereby informing and monitoring prevention and public health strategies and policies. As with new HIV diagnoses surveillance, changes in the absolute number and proportion of recently acquired infections detected through the RITA programme will need to be interpreted alongside any changes to testing patterns and reporting delays.

The availability of quality of epidemiological data and biomarkers is a key consideration in the planning of RITA as part of a HIV new diagnoses surveillance scheme. In addition to basic demographic (for example age, sex, region) and risk factor information, the availability of the following will be an important consideration in the design of the RITA system:

- RITA assay used: type of RITA test
- Setting: regional/national surveillance or other setting
- The predominant HIV subtype in the population, which may influence the type of essay used
- Testing strategy: unlinked or linked, named or anonymous
- Population and sample size, for example all newly diagnosed persons by risk group; knowing the number of negative as well as positive tests performed on the population of interest will influence the statistical method employed to calculate incidence
- Case information such as CD4 count and AIDS at diagnosis, previous positive test, and treatment history can be used to identify cases falsely classified as recent by the assay (see Section 3.3.2)
- Case information on testing history (in particular previous HIV negative test and evidence of recent seroconversion) can be used to identify cases falsely classified as long standing. This information is also important in incidence calculations where the total number of persons tested is unavailable (see Section 3.3.2)
- Additional variables to reflect the local epidemiology (such as ethnicity and date of arrival in the country)
- Presentation of results: overall proportion appropriately stratified, rate, incidence estimate, and associated confidence intervals
- The extent to which the findings are generalisable.

Other factors that may influence the interpretation and comparability of RITA results should also be considered:

- Testing patterns: proportion of first time and repeat testers, as well as testing intervals
- Under-reporting of new HIV diagnoses
- Missing patient-level data/reporting completeness
- Comparability of different RITA window periods, where different platforms are used.

3.11 Integration of RITA as part of routine HIV surveillance

The EU/EEA, thanks to its comprehensive surveillance systems for new HIV diagnoses, is in a unique position to incorporate RITA testing as part of routine HIV surveillance and produce comparable findings at the European and country levels. In addition to a large sample size, this approach provides greater representativeness of the HIV population. However, there will be challenges in integrating RITA with case reporting systems. Traditionally, HIV incidence estimates using RITA studies have relied on the availability of data on the total number of tests performed among the study population (both HIV negative and positive). These data are not routinely collected as part of HIV surveillance systems. New statistical methods to estimate HIV incidence on newly diagnosed populations have recently been developed in the US and France. These methods make it necessary to understand the extent to which those who undergo HIV tests are representative of the populations from which they are drawn; they also require an understanding of testing patterns and the overall size of the at-risk population. It is recognised that these data may not be readily available, may not be complete, and difficult to obtain in many countries.

4 Technical guide for integrating tests for recent infection as part of routine HIV surveillance

The aim of this chapter is to provide a practical step-by-step guide on how to integrate RITA as part of routine surveillance and estimate HIV incidence from case-based surveillance data.

This Chapter provides guidance on preparing the epidemiological and clinical data necessary for the algorithm; on how to performing testing, measuring and adjusting for misclassification; on estimating numbers and proportions of RI; and how to convert these data into population-based incidence estimates.

This document complements the available guidance provided by the WHO [10] on applying RITA to estimate HIV incidence from cross-sectional studies by supplying supplementary information specific to surveillance systems in European countries.

This technical guide is aimed at persons who are involved in the decision-making and implementation process of integrating RITA into their established HIV monitoring systems. Despite the fact that statistical methods are continuously evolving and that there are few data concerning the performance of the assays, this Chapter attempts to provide insights into the methods currently applied by countries with RITA already integrated into their HIV surveillance systems. This guide may be used by countries considering the implementation of RITA and need information regarding data and samples to collect.

The purpose of this chapter is to:

- provide guidance on country-level, case-based surveillance of recently acquired HIV infections;
- propose standardised, harmonised data collection systems to collect adequate and comparable data for estimating the proportion of recently acquired HIV infection in the EU; and
- work towards standardised approaches for RI testing and statistical methods for HIV incidence estimates.

4.1 Setting up a surveillance system: overview of implementation phases

Most European countries have HIV surveillance systems in place to monitor the number of new HIV diagnoses. The proposed steps for the integration of RITA are outlined in Figure 2.

Figure 2. Stages for the integration of RITA into routine HIV surveillance

1. Review the minimum data and specimen requirements for RITA and population-based incidence estimates and assess if it is feasible to obtain these data.
2. Review ethical frameworks; ensure requirements are/can be met.
3. Choose an assay and procure necessary laboratory equipment.
4. Train staff and ensure a diverse group of professionals form part of the RITA surveillance team.
5. Validate the assay locally (in-house and field).
6. Undertake a pilot study and monitor and evaluate the testing and data collection process.
7. Roll out sample and data collection nationally.
8. Perform analyses of programme coverage and representativeness, proportions of RIs and HIV incidence.
9. Report results.

The required investment in logistics and resources for the implementation of the surveillance of likely incident infections using biomarker assays will vary between countries, depending on the existing public health and laboratory infrastructures. On completion of the planning stage, countries with a small annual number of new diagnoses may choose to review whether this incidence estimation approach is cost effective. Cross-sectional surveys should be considered next to other incidence estimation methods such as back-calculation approaches.

4.2 Implementation team

Ideally, a variety of specialists from diverse disciplines need to become engaged to form an advisory and/or steering committee group. Ideally the team will consist of:

- a consultant microbiologist;
- a sexual health physician;
- representatives from public health departments;
- representatives from NGOs and community outreach organisations;
- laboratory scientists and technicians;
- a consultant epidemiologist;
- a statistician;
- a data manager;
- an administrator.

Terms of reference and roles for each team member should be developed. Issues to consider are:

- the development of the surveillance system and the legal/ethical framework;
- costs;
- data protection;
- the timely reporting of surveillance data results;
- quality assurance and provision of transparent processes and procedures; and
- the development of standard operating procedures (SOPS).

4.3 The Recent Infection Testing Algorithm

There are numerous RITAs which depend on the number and type of assays and clinical information incorporated. For all RITAs, the duration of the mean recency period and the FRR must be known. The FRR may be derived locally in the population studied, or a literature value can be applied.

The most basic algorithm involves testing a sample from an individual newly diagnosed with one TRI assay and then classifying the infection as 'recent' or 'long-standing' based solely on the result of the assay. It is important that the sample is taken close to the date of the HIV diagnosis because longer intervals make it less likely that an infection will be classified as recent. Ideally, the TRI is performed on the diagnosis sample and we recommend that samples taken more than four months after the diagnosis date should not be included in analyses.

As current TRIs produce false recent results among individuals on ART or with an AIDS-defining illness, the following increasingly complex algorithms enable more refined estimates and should be used if the data and resources are available. Clinical data which indicate a probable long-standing infection will reduce misclassification rates. The following types of data can be incorporated:

- CD4 count: A low CD4 count is indicative of advanced-stage HIV infection; studies have shown that the FRR is higher among persons with low CD4 counts (<200cells/mm^3); to reduce the FRR, a RI result may be reclassified as long-standing among persons with low counts. Occasionally, a low CD4 reading can be observed shortly after infection acquisition, however the probability is much higher that a low CD4 count at diagnosis is a consequence of an established infection.
- AIDS defining illness: The presence of an AIDS-defining illness is highly indicative of a long-standing HIV infection. False recent rates can be reduced by reclassifying cases of RI among persons who develop an AIDS-defining illness within a year of their initial diagnosis.
- ART: As with low CD4 counts, high FRRs have been observed among persons receiving ART. It may occur that individuals newly diagnosed with HIV may have been on treatment (pre- or post-exposure prophylaxis). The FRR can be reduced by reclassifying RI diagnoses to long-standing infections among those on ART before the sample was tested.
- Viral load: Undetectable or low viral load can result from natural suppression or may be indicative of ART treatment. False recent rates are higher among those with a low viral load. Reclassifying recent cases as long-standing among those with VL< 400 copies/ml will reduce FRRs.

The more information is incorporated into the algorithm, the lower the expected misclassification rate.

Figure 3. Recent Infection Testing Algorithms, required and optional components

Required	1. Confirmed HIV-positive sample, taken within three months of the diagnosis date.
Required	2. Sample tested with one or more assays and results linked to a new HIV diagnosis report.
Optional	3. RIs reclassified as long-standing if the individual has an AIDS-defining illness.
Optional	4. RIs reclassified as long-standing if the individual is receiving or has previously had ART.
Optional	5. RIs reclassified as long-standing if the individual has CD4<200cells/mm^3.
Optional	6. RIs reclassified as long-standing if the individual has a viral load <400 copies.

If possible, two different assays should be applied; RIs are where both assays classify the sample as recent. This may be an approach to reduce the FRR where limited clinical information is available. The lowest FRR is expected where multiple types of assays are used and all clinical information is incorporated into the algorithm. However, the evidence base for this is continuously developing, and the latest available information should be considered when deciding which components of the algorithm should be incorporated in order to lower FRR. Including information on the dates of the last negative test can also reduce misclassification because RIs are confirmed if these dates are within the recency period. As outlined in the WHO guidance document, it is unlikely that any combination of assays or clinical information will eliminate misclassification completely; therefore, it is necessary to estimate the FRR for the specific algorithm applied.

4.4 Minimum data fields essential for RITAs

Essential country-level data which must be available for the implementation of RITA surveillance are:

- number of new HIV diagnoses reported per year, stratified by gender and risk group;
- highly complete, individual-level datasets;
- essential individual-level data which must be collected:
  - date of first HIV-positive test;
  - result of one or more RI tests and the date the sample was taken.

If available, other data to be collected to refine the algorithm (see Section 4.6) are:

- CD4 cell count and the date of count;
- clinical stage (e.g. AIDS);
- ART history and date of treatment initiation;
- viral load and the date of the viral load measurement.

Most countries in Europe collect new diagnoses and risk group data. The availability of CD4, AIDS, ARV history and viral load data will vary depending on the design of the surveillance system and the routines to report these data. A sample data dictionary and schematic of the data flow in the UK is provided in Annexes 6 and 7.

4.5 Minimum data fields essential for population-based incidence estimates using RITAs

Essential data for converting RITA into population based incidence estimates are:

- individual background information on HIV testing patterns (previous HIV test, date and result);
- population background information on HIV testing patterns;
- population background information on HIV subtypes;
- sources of data for population size estimates of subgroups/most-at-risk populations (MARP), e.g. estimate of MSM population.

To perform population-based incidence estimates, individual-level testing data are essential for weighting the observed number of RIs. Those who appear to have recently acquired their infection are categorised as first-time and repeat testers, based on these information. Testing data may be extracted from patient records or through linkage to another surveillance system, for example one that collects sexual health clinic attendance, testing and diagnosis data.

Currently, all available assays have been validated only for HIV-1 and it is therefore essential to have an estimate of the baseline prevalence of subtypes. To obtain rates, denominators are needed of population estimates of the various subgroups. Background data on general population HIV testing patterns, the prevalence of HIV subtypes and the size of most-at-risk populations may also be derived from testing data within sexual health clinic networks, or community surveys, and if available, national representative surveys, for example the UK National Survey of Sexual Attitudes and Lifestyles (NATSAL) and the French National Random Probability Survey of Sexual Behaviours (CSF).

4.6 Data collection

The main objective of data collection is to obtain detailed, accurate and complete information. Most countries have surveillance systems in place that monitor the number of HIV diagnoses and report annually. These data should be used as a basis for the algorithm and the HIV incidence estimates. Recent infection testing data will be generated by the centralised or decentralised laboratories enrolled in the programme and should be submitted to the data manager who will link these to new diagnoses reports.

There may be numerous sources from which the additional information required for RITA and HIV incidence estimates can be obtained, including patient records, laboratory reports and other surveillance system databases. Data sources for individual-level data may often be mixed; in order to maintain clear information pathways, the sources of variables should be recorded and original data submission forms kept. Information may be duplicated by the use of multiple sources and can, in some instances, be inconsistent. For example, a patient may report no previous tests but information in a database indicates otherwise. Decisions on which data sources to prioritise should be consistent throughout.

Data entry, coding and quality

We recommend that standard procedures be followed for data entry and validation, thus ensuring high data quality. A sample data dictionary and coding scheme can be found in Annex 6.

Data linkage

Depending on the design of existing surveillance systems, one or more data linkage steps may be necessary to combine all the variables required for the algorithm (ensure that this process does not breach any ethical frameworks). This could be challenging if individual-level identifiers vary across surveillance systems. Individuals can be linked across databases with the use of algorithms beginning with strict conditions that link only when there is a high level of certainty that cases refer to the same individual. Should matching levels be low, the conditions may be relaxed to increase the proportion that will be linked. Combinations of variables to create 'pseudo-identifiers' may include:

- clinic/site ID
- patient ID
- date of birth (DOB)
- date of diagnosis
- gender
- soundex
- regional information

For surveillance purposes, a certain margin of error introduced by the linkage is acceptable and in many cases inevitable. Should the RI results at any point be returned to the patient, it is crucial that only strict matching conditions are used.

4.7 Data sources for population estimates

In order to present data as incidence rates, the total number of people at risk of HIV needs to be known. Therefore, data sources need to be found which make it possible to estimate the size of certain subpopulations, for example MSM or those who are sexually active and/or inject drugs.

Estimates of the size of MARPs may be obtained from census data, nationally representative surveys, national sexual health surveys, anonymous testing, and community-based behavioural studies. Availability and sources of data will vary between countries. If data are not available, population sizes may be estimated. Capture–recapture or multiplier methods can be applied to derive these. Methods to estimate population size have been described by a UNAIDS/WHO working group [65] and by Thompson [66].

There is a tendency for countries to initiate recent infection testing and incidence estimations for high-risk groups such as MSM. The information required for these estimates is the same as for general population estimates. For example, the size of the MSM population will need to be known alongside the number diagnosed. A considerable

limitation of this approach is the smaller number of diagnoses and the impact on the accuracy of incidence estimates.

4.8 Biomarker assays for recent infection testing

Annex 2 describes various types of assays for RI testing. There is a range of assays able to measure the stage of an immunological response to an infection with HIV. These are:

- less sensitive EIA (measures antibody titres, e.g. Vironostika-LS);
- proportional assay (measures the proportion of HIV-specific IgG compared with total IgG e.g BED CEIA);
- avidity index (measures the strength of the antibody-antigen bond, e.g. AxSym avidity);
- relationship (immunodominant) assay (measures the relationship between HIV-1 glycoproteins and V3, e.g. IDE-V3);
- line immunoassay (is a method similar to Western blot which uses synthetic oligopeptides and recombinant antigens, e.g. INNO-LIA HIV I/II Score); and
- IgG3 Anti-HIV (measures the IgG3 response to p24Ag, which typically is only detectable for the first 1–4 months.

A list of suppliers can be found in the appendix of a WHO document [10]. Further information regarding the characteristics of these assays is available on the CEPHIA website (http://www.incidence-estimation.com).

Deciding on which is the most appropriate assay to use may depend on the outcomes of the CEPHIA project, which aims to better describe (for 10 different assays) the mean duration of recency and the FRRs. In addition, the choice of assay will be guided by the type of sample a country collects (some countries may have access only to dry blood spot specimens while others are able to collect serum), the commercial availability of the assay, the costs, any existing regulatory frameworks restricting the testing of particular specimens or use of assays, and equipment. Irrespective of assay type, the process for implementing testing will be the same and will include:

- choosing a supplier, purchasing the equipment and consumables;
- collecting samples with which the equipment can be validated/calibrated;
- developing protocols for training and testing (including sample storage and transportation);
- training staff; and
- developing a quality assurance process and protocol.

If countries that already conduct RI testing, decide to switch the equipment/assay, the new equipment/assay will need to be calibrated against the old by retesting previous specimens. Similarly, if multiple laboratories are to undertake testing, even minor changes in the sampling or testing processes may result in inconsistent results between laboratories. Protocols need to be developed which outline the procedures in detail, including procedures on sample storage, transportation and testing. Information specific to each assay is available on the CEPHIA website, in addition to CEPHIA's standard operating procedures for tests.

To ensure testing quality, it is necessary to conduct assessments at regular intervals. We recommend taking part in quality assurance schemes, following minimum sample quality standards, monitoring and servicing equipment, and running controls.

The use of one specific assay and/or algorithm for all European Member States may be proposed in the future in order to enable improved cross-country comparisons of incidence estimates. However, this depends on the identification of the best performing assay and the assessment of its applicability across Europe.

4.9 Local assay validation, estimating FRRs and mean duration of recency

It is recommended that all used assays are validated locally in the population of interest in order to more accurately determine the parameters for incidence estimation. FRRs are established by examining assay results among individuals known to have been infected for longer than a time T post infection (conveniently set to a year). For example, if samples are tested from 1000 individuals who have been known to have been infected for more than a year and 60 infections appear to be recent, the FRR would be 6% (95% C.I. 4.61–7.66) (a further example is available from http://www.incidence-estimation.com/uploads/Calibrationv0.5.xls). This can be reduced if those known to be on ART and those with evidence of late-stage disease due to the presence of an AIDS-defining illness are excluded [67]. It is important that the exclusion criteria applied to the RITA are the same as those used to assess the FRR. Therefore, well-characterised samples from individuals known to have a long-standing infection are needed. The more information is supplied with the samples, the better the FRR can be characterised with stratified analyses. For example, in some populations it has been shown that older age is associated with higher FRRs [68].

To establish the mean recency period, samples from a prospective cohort of seroconverters are needed for which the date of seroconversion is known or can be estimated to a reasonable level of accuracy. Testing must be undertaken at regular intervals to plot the assay results over time. Braunstein et al. have performed this for both the BED CEIA and the AxSym avidity assay in a single cohort, and Bharat et al. have undertaken a larger study measuring the BED CEIA assay among samples from 17 cohorts [69] [68]. In order to estimate the recency period, the mean duration at which individuals cross a given cut-off value (e.g. an avidity index of 0.80 for the AxSym avidity) is observed. Sweating et al. have described two further statistical approaches to estimate this period for the AxSym avidity assay. Their method accounts for the interval-censored nature of both the date of seroconversion and the date of crossing a specific threshold [70].

In some cases, existing data may be used to make estimations. For example, data may be available from individuals who get frequently tested at sexual health clinics, so a seroconversion date can be established with considerable accuracy. Further, residual specimen from subsequent samples taken for standard baseline tests such as CD4 and viral load measurements may exist. If enough of these can be recovered, this may be a more cost-effective and practical way to assess the mean recency period in the population to be studied. Kassanjee et al. undertook a study examining the mean recency duration and FRR among repeat blood donors who were observed to seroconvert [71] [72].

If data are not available, an externally derived FRR and mean recency period can be used. These values are published in peer-reviewed literature or, if the chosen assay is one of the 10 reviewed by the consortium, on the CEPHIA website (http://www.incidence-estimation.com).

4.10 Piloting testing and data collection

The complete testing, data collection and linking process should be trialled before it is rolled out on a national scale to identify any potential glitches. All members of the implementation team should be involved in the review process.

4.11 Missing data

Across all surveillance systems, missing data cannot be completely avoided. For each of the most important variables, the proportion of missing data should be assessed, and established if these are associated with specific variables. The extent of missing data should be reported. Rather than performing analyses on subjects with complete data (complete-case analysis), with the consequence of losing statistical power and implicitly assuming that subjects excluded are equivalent, a better approach is to apply imputation techniques. These use auxiliary variables to complete data and allow quantifying the uncertainty associated with the imputation process. For the estimation of population-based HIV incidence in France and the US, multiple imputation procedures were used [21].

4.12 Estimating population-based HIV incidence

With a complete dataset, the algorithm can be applied for the final classification of infections as 'recently acquired' or 'long-standing' . When presenting the results, it is good practice to report the number of cases that were excluded/reclassified at any stage. The following example assumes that CD4, ART and AIDS data were incorporated in the algorithm: If, based on the assay, 300 cases appeared as recent and 20 had a CD4 count of less than 200 cells/mm^3, eight had been on ART, and six had AIDS-defining illnesses, the total number of cases classified as 'recently acquired' would be 268 cases.

With an estimate of the mean RITA duration (or the distribution of these durations in the population) and information on testing history, the principle of the model developed by Karon et al. can be described as follows:

HIV diagnoses are stratified into subpopulation groups according to demographic variables to constitute homogenous risk groups. The model considers the diagnoses observed as RIs as a random sample, drawn from the population of HIV infections occurring within one year (incident cases). The corresponding sampling probability is calculated as the probability of being tested within one year following infection (p_1) times that of being detected as recently infected while diagnosed within a year of infection (p_w). The first probability p_1 is estimated separately among individuals who report a negative test before diagnosis (repeat testers) and those diagnosed at their first test (new testers). These conditional probabilities are primarily determined by testing history (p_1 for repeat testers), the proportion of HIV infections diagnosed at AIDS stage (p_1 for new testers) and the duration of recency (for p_w). The whole distribution of the duration of recency can be used instead of the mean [73]. The number of diagnoses observed as RIs can be corrected by the FRR as proposed by Le Vu et al. [72] The number of incident cases within a subpopulation is then derived from the size of the sample of RIs divided by the sampling probability ($p_1 * p_w$). Estimates should be presented as person-years.

The principle aim and output of the programme should be to estimate HIV incidence. However, in the absence of incidence estimates, proportions of RI may be presented according to MARPS, stratified by age, region and other relevant parameters. An example of this is shown in Annex 8. Semaille et al. have presented such data by

examining predictors of RI in France [23]. It is important to note that the proportion of RI among those who were newly diagnosed with HIV does not reflect incidence as it does not take into account the undiagnosed population. Proportions must be interpreted alongside programme coverage and HIV testing patterns.

4.13 Limitations

Several limitations concerning the use of a RITA to estimate incidence have already been highlighted in this document. In summary, the following aspects determine how robust the final incidence estimates are:

- under-reporting of new diagnoses
- the quality and completeness of data
- how well testing data reflect testing patterns
- how well the assays have been characterised for the population of interest
- the accuracy of data estimating population sizes.

A higher number of diagnoses will generate narrower confidence intervals of the estimates, enhancing the ability to detect significant changes in incidence over time.

It is essential that all limitations are acknowledged in the reporting of data, which may be presented either in national HIV reports or peer-reviewed publications and can be used as a basis for policy development. To facilitate the comparison of methods, RI data should be presented by risk group and incidence estimates by person-years.

4.14 Returning results to the patient

In the UK, TRI results have been of value in clinical settings, allowing patients and clinicians to discuss results alongside other routine baseline tests. Two recent studies found that patients valued information about the likely timing of their infection, and no negative outcomes were experienced by those receiving these results [74] [75]. Another study indicated that clinicians found the results helpful and were comfortable discussing these with patients, particularly in the context of possible HIV seroconversion illness [76]. Work is ongoing to evaluate the role of RITA as a tool to enable accelerated partner notification.

5 Conclusions

The development of RITA assays and their use in different epidemiological studies has provided a practical alternative to lengthy, expensive, and logistically challenging cohort studies. Published incidence studies using RITA assays produced a wide range of HIV estimates in key populations. Many estimates were difficult to compare due to a lack of consistency in the study design, biases in the selected populations, small sample size, different types of assays, and their way of presenting the major findings. These issues underscore the need for a concerted, standardised approach for monitoring recently acquired HIV infections as a proportion of all new diagnoses. It also makes a point for estimating HIV incidence across Europe.

To date, RITA testing in developed countries has been largely performed to estimate HIV incidence among sentinel clinic attendees, as part of a cross-sectional or cohort study focused on risk populations. An increasing number of countries have also implemented RITA testing as part of their national surveillance.

Each epidemiological approach has certain advantages and disadvantages. Data obtained through a sentinel surveillance system are generally less expensive than active surveillance of the total population. In addition, data are of higher quality than those provided by passive systems. However, the main limitation is to ensure the representativeness of the sample, particularly if recruitment is in a clinical setting, where there may be an over-representation of people seeking care, resulting in a potential upward bias in incidence estimate. Similarly, cross-sectional studies of hard-to-reach populations often rely on a convenience sampling.

Limited data on the false recent rate (FRR) are available for the various RITA platforms currently in use, with estimates ranging from ~1% to ~5%. Assuming that these results are typical, a RITA assay will require a large number of well-characterised samples from long-standing infections to reliably establish an FRR in a given population. Such samples may be logistically difficult to obtain and costly to test. Data collected as part of a routine national or regional surveillance system will, at the very least, identify those individuals who have a previous positive HIV diagnosis and can thus be used to reduce the FRR. In addition, other case-based data such as CD4 cell counts, AIDS diagnoses, and ARV experience can also be used to minimise the FRR, provided such data are available.

Other factors that influence the interpretation and comparability of RITA results should also be considered: under-reporting of new HIV diagnoses; missing patient-level data/reporting completeness; comparability of different RITA window periods, where different platforms are used for certain HIV subtype as this may affect the performance characteristics of the RITA test.

Methods to estimate incidence depend on the availability of data on HIV-negative tests for the studied populations. Where relevant information is available – or can be estimated from alternative data sources (including community surveys, laboratory databases, or STI clinics –, the Janssen formula or similar approaches may be used. However, many national surveillance systems do not routinely collect these data, and new statistical methods have therefore been developed to incorporate HIV testing histories (e.g. last negative) as a proxy measure. The challenge is to ensure that this information is sufficient to provide robust estimates.

Importantly, the WHO working group on HIV incidence assays calls for better evaluation data to guide the selection use and interpretation of optimal RITA assays. This can only be achieved through a concerted effort involving public health agencies in partnership with private industry.

To address the selection bias inherent in other epidemiological approaches and to ensure greater comparability across EU/EEA countries, RITA testing should be used for all newly diagnosed infections. Monitoring recent HIV infections at the EU/EEA level and facilitating the comparison of trends in incidence between countries requires consistent and transparent methodologies, with a harmonised presentation of results.

RITA testing can be incorporated into the routine national HIV surveillance system using the technical guide supplied in this document in order to achieve standard outputs, provide a sustainable approach, and offer valid results.

Ideally, a common assay is to be applied in all European countries. Outcomes of the CEPHIA project will shed light on whether this is possible.

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Annex 1: Glossary

Recent Infection Testing Algorithm (RITA)
A laboratory test or combination of tests, or combination of tests and supplementary laboratory and clinical information, used to classify an HIV infection as recent or not recent.

Recently acquired HIV infection
A transient state that occurs for a period soon after HIV exposure. Its duration varies between individuals and depends on the method used for its detection.

HIV incidence
The number of new HIV infections occurring in a population, usually expressed as a rate of infection per person per unit time (e.g. 'infections per 100 person-years').

Assay or test for recent HIV infection
A laboratory test used to classify a case of HIV infection as recent or not recent.

Assay-defined recent HIV infection
The phase of infection from HIV seroconversion until the end of the assay-defined incidence window period.

Assay-defined long-standing HIV infection
The phase of infection following the assay-defined incidence window period.

Incidence assay window period
For a given HIV incidence assay, the duration (as determined by specific laboratory parameters) of the period defined as recent HIV infection by the assay.

Seroconversion window period
The duration of the period from the time of HIV infection until the first detection of HIV antibody response by routine serologic assays (e.g. EIA, rapid tests).

Window period
Window period of HIV incidence assay is estimated by using seroconversion panels to define the assay cut-off and the associated mean and standard error.

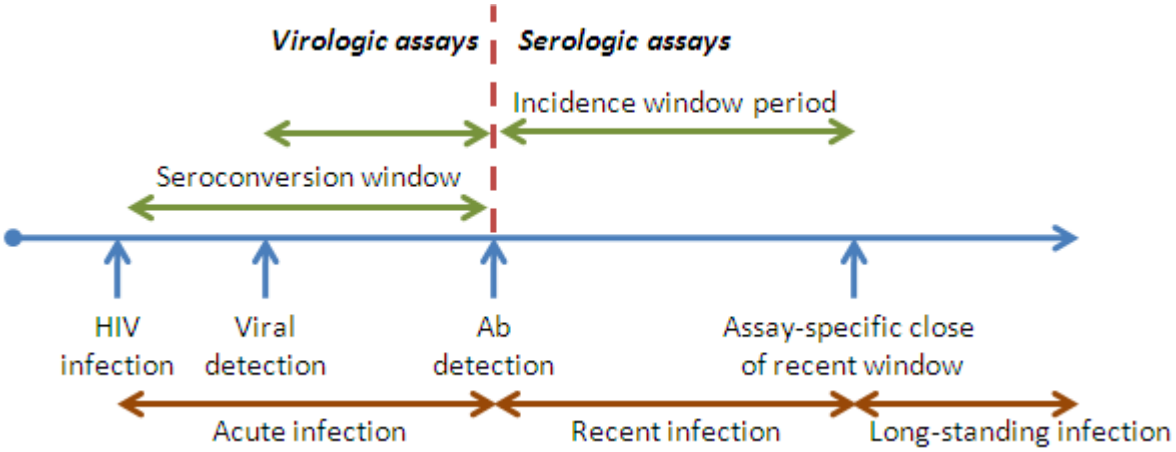
RITA false recent rate (FRR)
The fraction of non-recent HIV infections in a population that is incorrectly classified as being recent by the RITA. This parameter is essential for estimating HIV incidence with a RITA, but cannot generally be provided by the assay developer or manufacturer, as it depends on characteristics of the tested population.

RITA specificity
The fraction of non-recent infections that are correctly classified as recent.
RITA specificity = 1– RITA FRR

Mean RITA duration
The mean duration of recent HIV infection in a population of people with HIV infection. This parameter is essential for the estimation of HIV incidence using a RITA and is determined by the assay developer or manufacturer. Ideally between four to eight months, the mean RITA duration can vary according to the specific RITA being used, and each RITA may vary by HIV subtype. A RITA should not be considered for use in estimating HIV incidence in a population if its mean duration has not already been determined for that population.

Acute/early HIV infection
The phase of infection that occurs between the first detection of HIV by virologic assay (e.g. RNA or viral antigens) and the first detection of HIV antibody response by a serologic assay (e.g. EIA). These concepts are summarised in Figure 1-1.

Figure 1-1. Assay-defined HIV detection windows and infection periods



Source: 'Methodologic guidance for validation of existing and future HIV incidence assays', Version 5.0, 13 October 2008

Annex 2: RITA assays

Less-sensitive EIA (e.g. Vironostika-LS)
These tests are based on the principle that antibody titres increase for several months following infection. Confirmed positive sample are retested with an enzyme immunoassay (EIA) that is made less sensitive by dilution (1/20 000) and a reduced incubation time to identify samples of low anti-HIV antibody titre. Low antibody titre correlates with recently acquired HIV infection.

Proportional assay (e.g. BED-CEIA)
These assays measure the proportion of HIV-specific IgG and total IgG. The proportion of HIV-specific IgG increases with time, therefore those with a lower proportion represent recently acquired HIV infection.

Avidity index (e.g. AxSYM avidity)
The avidity refers to the strength of the association between the viral protein (antigen) and the HIV-specific antibody. Total anti-HIV response is compared with the anti-HIV response when incubated with a denaturing agent that separates weak affinity antibodies. This is used to calculate an avidity index that increases with time.

Immunodominant assay (e.g. IDE-V3 assay)
The IDE-V3 assay is based on two HIV-1 envelope glycoproteins (GP41 and GP120) that induce the most consistent antibody responses. Responses to these antigens are combined in a mathematical formula to distinguish recent from long-standing HIV infections.

Line immunoassay (e.g. INNO-LIA HIV I/II Score)
The line immunoassay is a similar to a western blot and uses various synthetic oligopeptides and recombinant antigens. The Inno-LIA is one such line immnoassay, the score of which can be used to distinguish recent from long-standing HIV infections.

Annex 3: Literature search and results

A systematic search of PubMed was undertaken, using a number of key terms. These terms were used to search MeSH terms, article titles, and abstracts. Below is a summary of the number of articles identified with each term individually, and in combination.

1.	"HIV"[Mesh]	65 932
2.	"incidence"[Mesh]	131 927
3.	"recent"[TI]	44 081
4.	"incidence"[TI]	61 003
5.	"incident"[TI]	3 759
6.	"STARHS"[TI]	5
7.	"RITA"[TI]	239
8.	"recent"[TIAB]	492 769
9.	"incidence"[TIAB]	398 118
10.	"incident"[TIAB]	29 545
11.	"STARHS"[TIAB]	31
12.	"RITA"[TIAB]	562
13.	1 AND 2	5 733
14.	3, 4, 5, 6 OR 7	108 807
15.	8, 9, 10, 11 OR 12	891 121
16.	1 AND 14	549
17.	1 AND 15	4 447
18.	1 AND 2 AND 14	281
19.	1 AND 2 AND 15	681

Using search criteria '19' as a basis, papers that were published prior to Robert Janssen's initial paper on the use of a modified Abbott 3A11 were excluded, as this review is limited to HIV incidence studies using RITA assays. This resulted in a final set of 441 HIV incidence-related publications, published between 1998 and September 2010. The full PubMed query is shown below:

'HIV"[Mesh] AND "incidence"[Mesh] AND ("recent"[TIAB] or "incidence"[TIAB] or "incident"[TIAB] or "STARHS"[TIAB] or "RITA"[TIAB]) AND 1998:2010 [dp]

Similar detailed searches of CINAHL and MEDLINE and Google Scholar, using the same key search terms, when compared with the 441 publications identified through PubMed, yielded no additional publications, or articles.

All 441 abstracts were scanned and shortlisted against the following criteria:

- Exclude validation and comparative studies (unless providing an estimate of HIV incidence among the study population)
- Exclude items that did not use RITA assays
- Exclude those studies among populations outside Europe, the US, Canada and Australia
- Exclude publications in languages other than English
- Exclude studies not providing estimates of incidence or proportion of incident infections.

Where papers could not be excluded based upon information provided in the abstracts, or where abstracts were unavailable, full papers were obtained. A final shortlist of 44 papers were identified and included in the review. All citations from the initial shortlist were reviewed to identify any additional publications not already included. This yielded an additional four papers, giving a total of 48 peer-reviewed articles. A targeted Google Scholar search yielded three additional conference abstracts and one poster, all of which were included in this report.

Annex 4: Recently acquired HIV infections in key populations

Figure 4-1. Young people

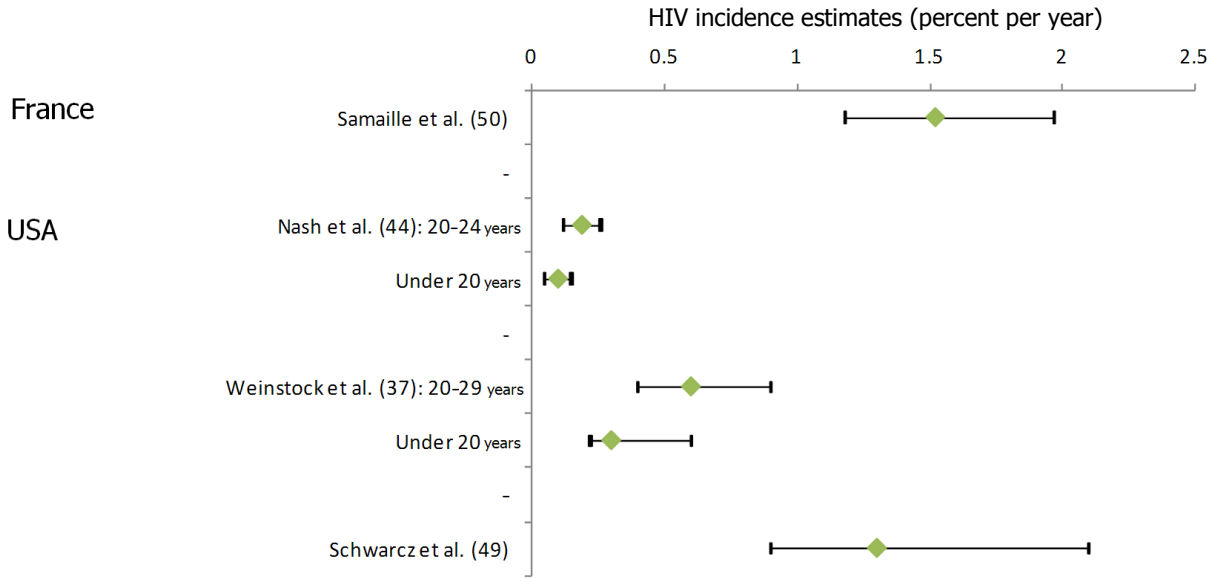


Figure 4-2. Young people

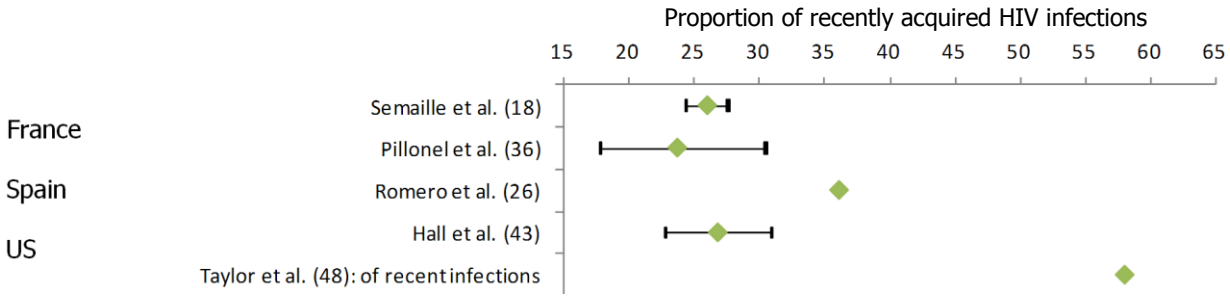


Figure 4-3. Young MSM

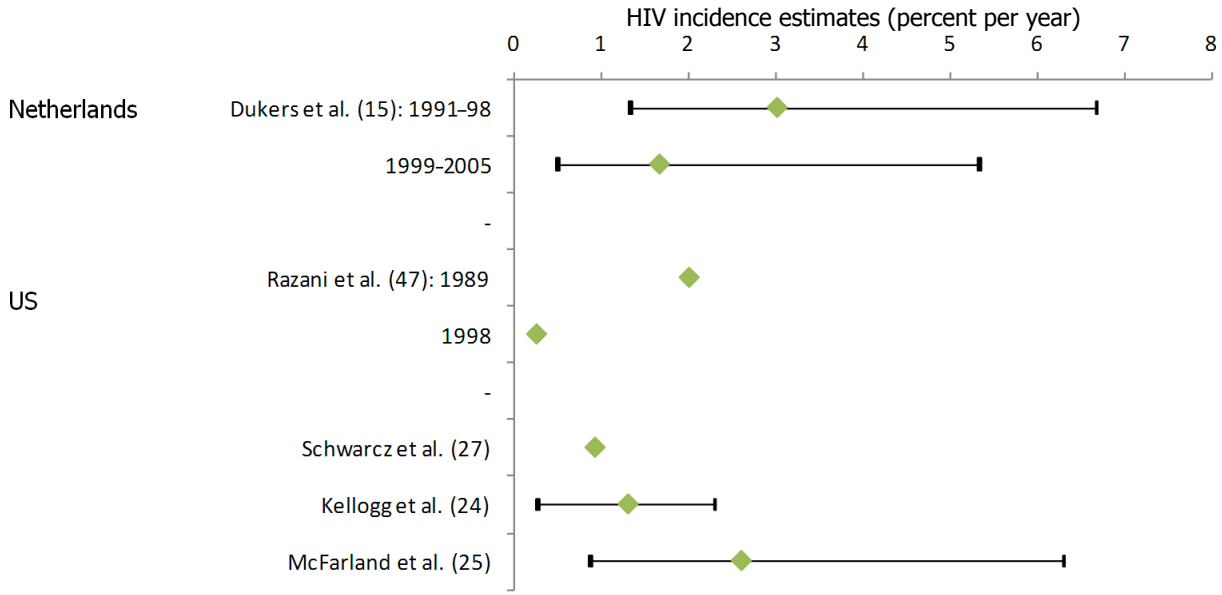


Figure 4-4. MSM (all age groups)

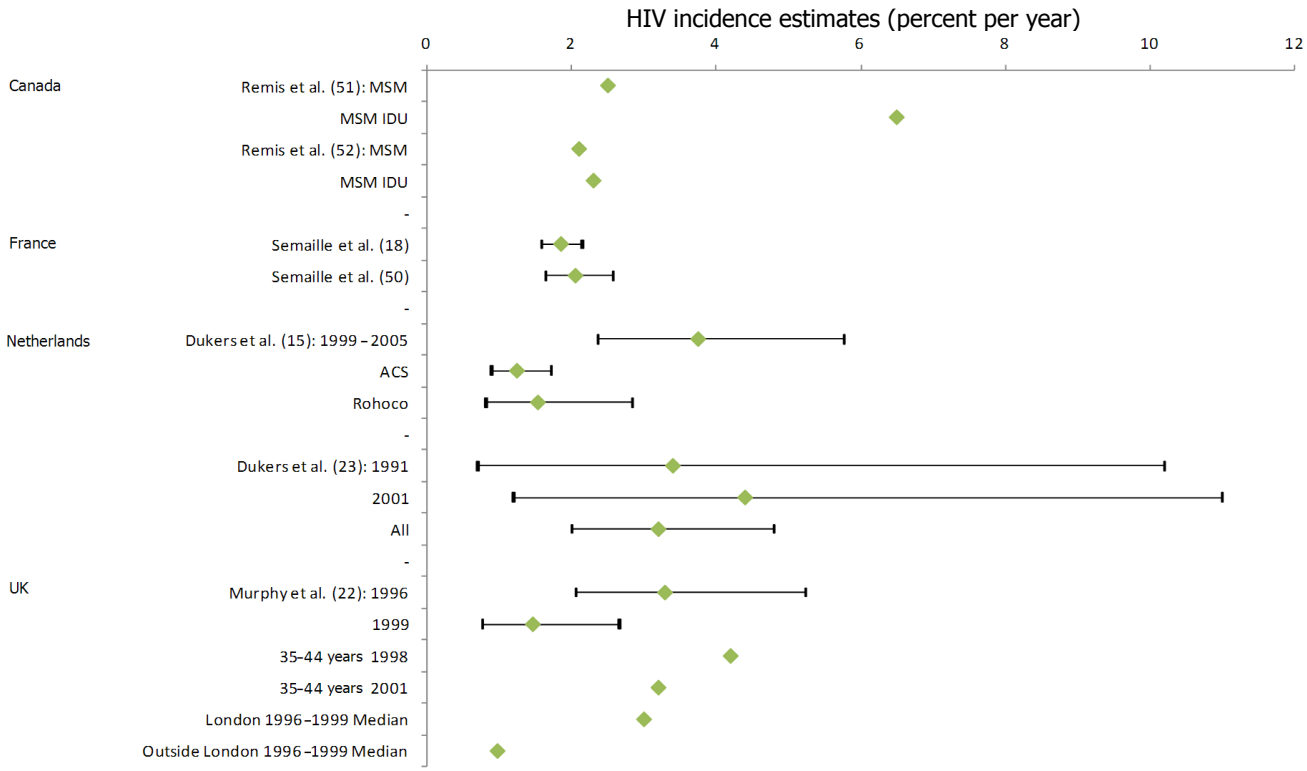


Figure 4-4. MSM (all age groups), *continued*

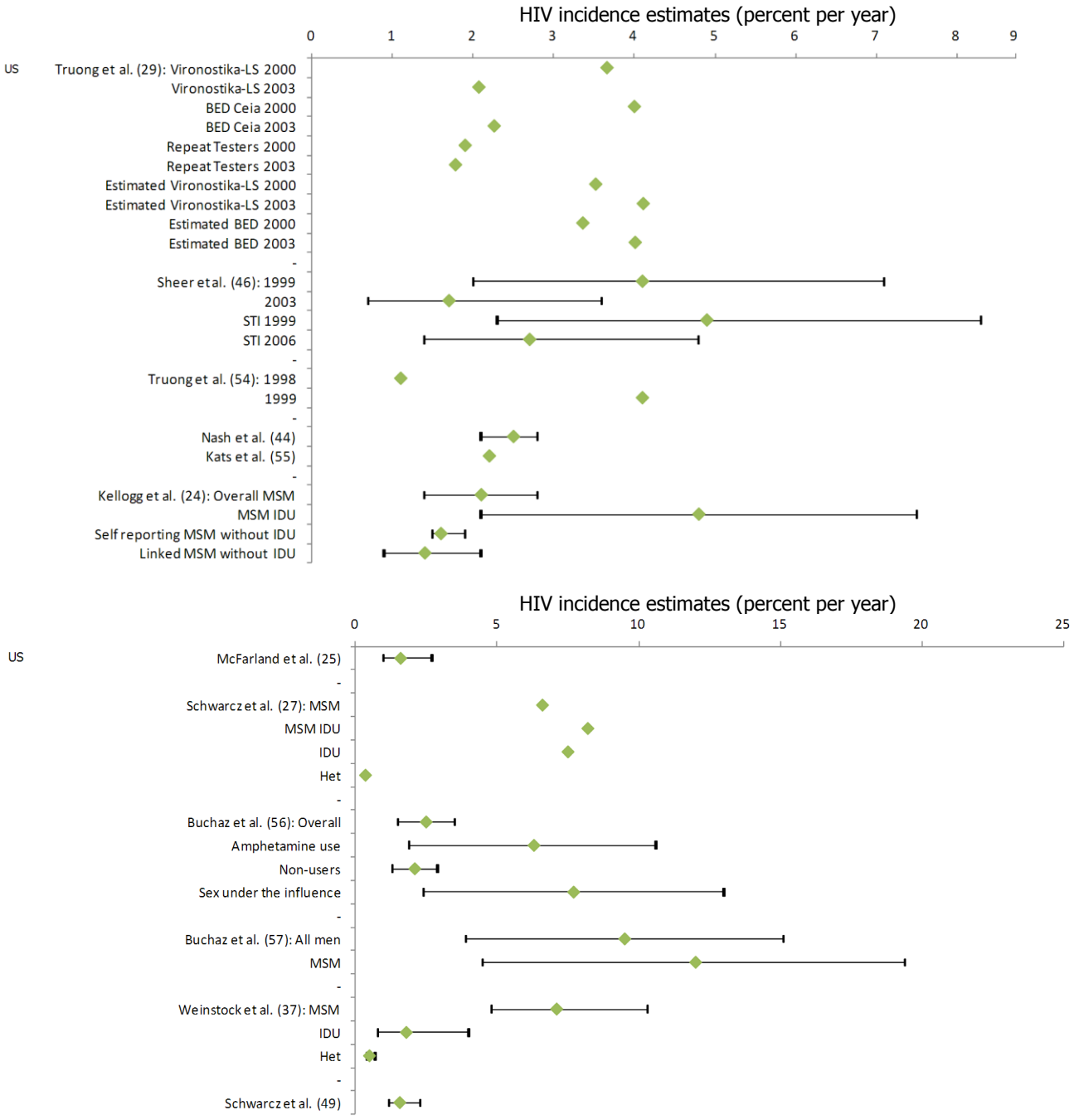


Figure 4-5. MSM (all age groups)

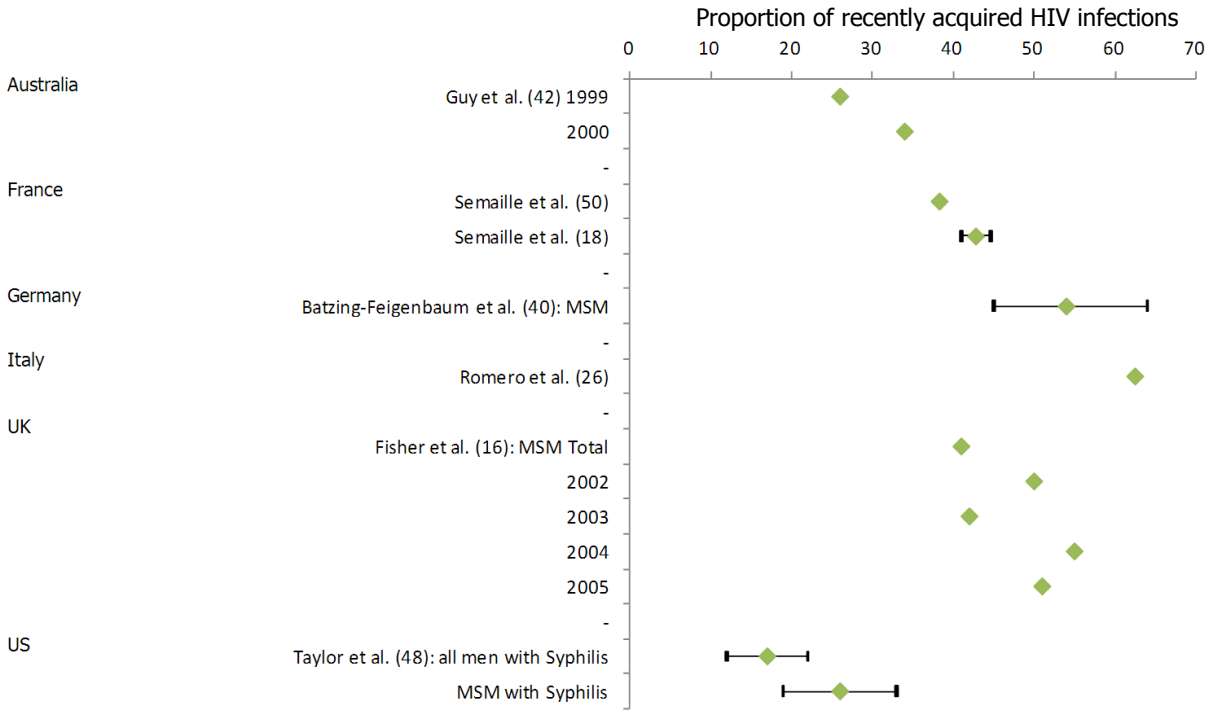


Figure 4-6. Injecting drug users

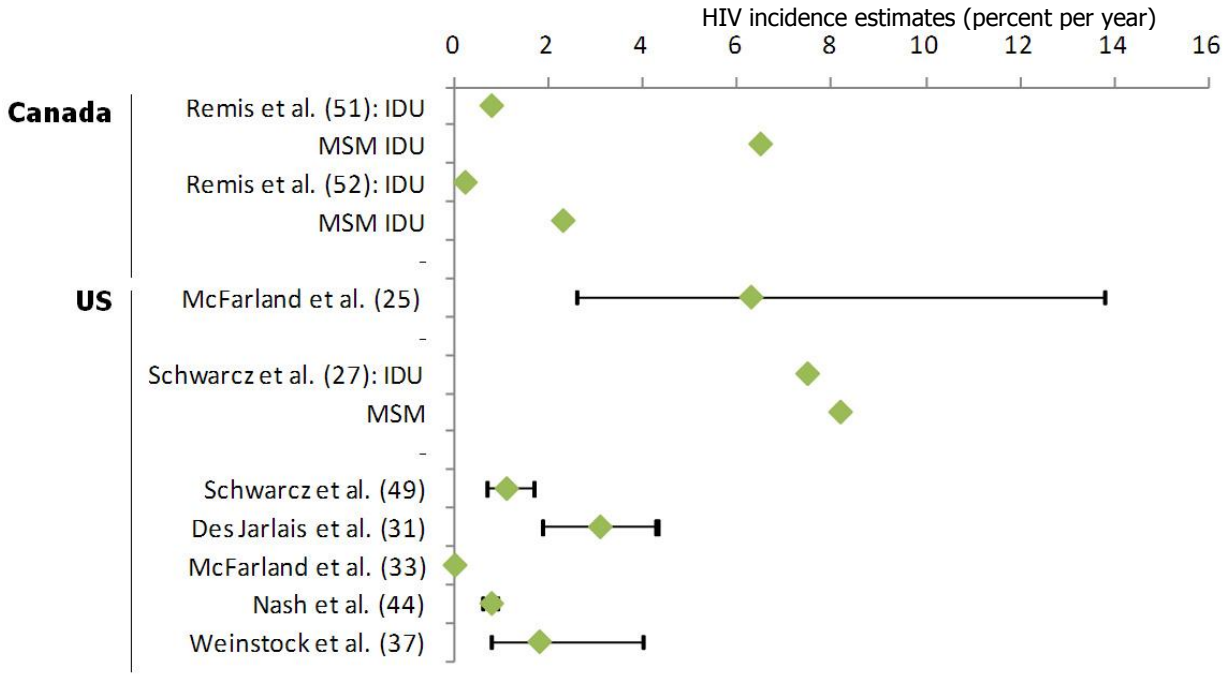


Figure 4-7. Injecting drug users

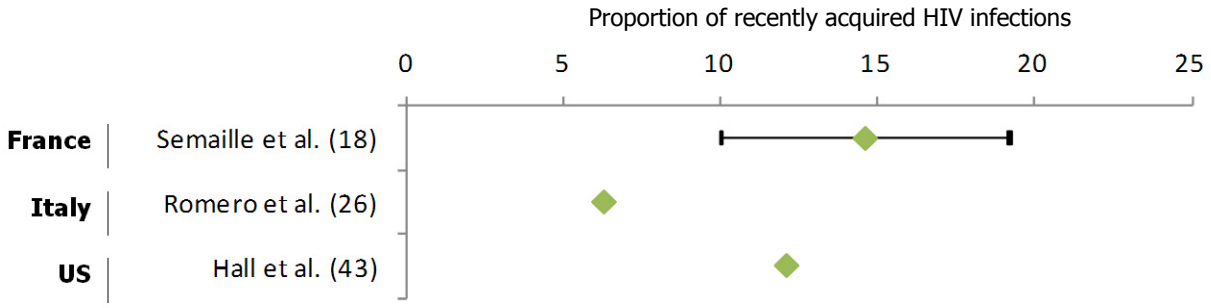


Figure 4-8. Migrants and ethnic minorities

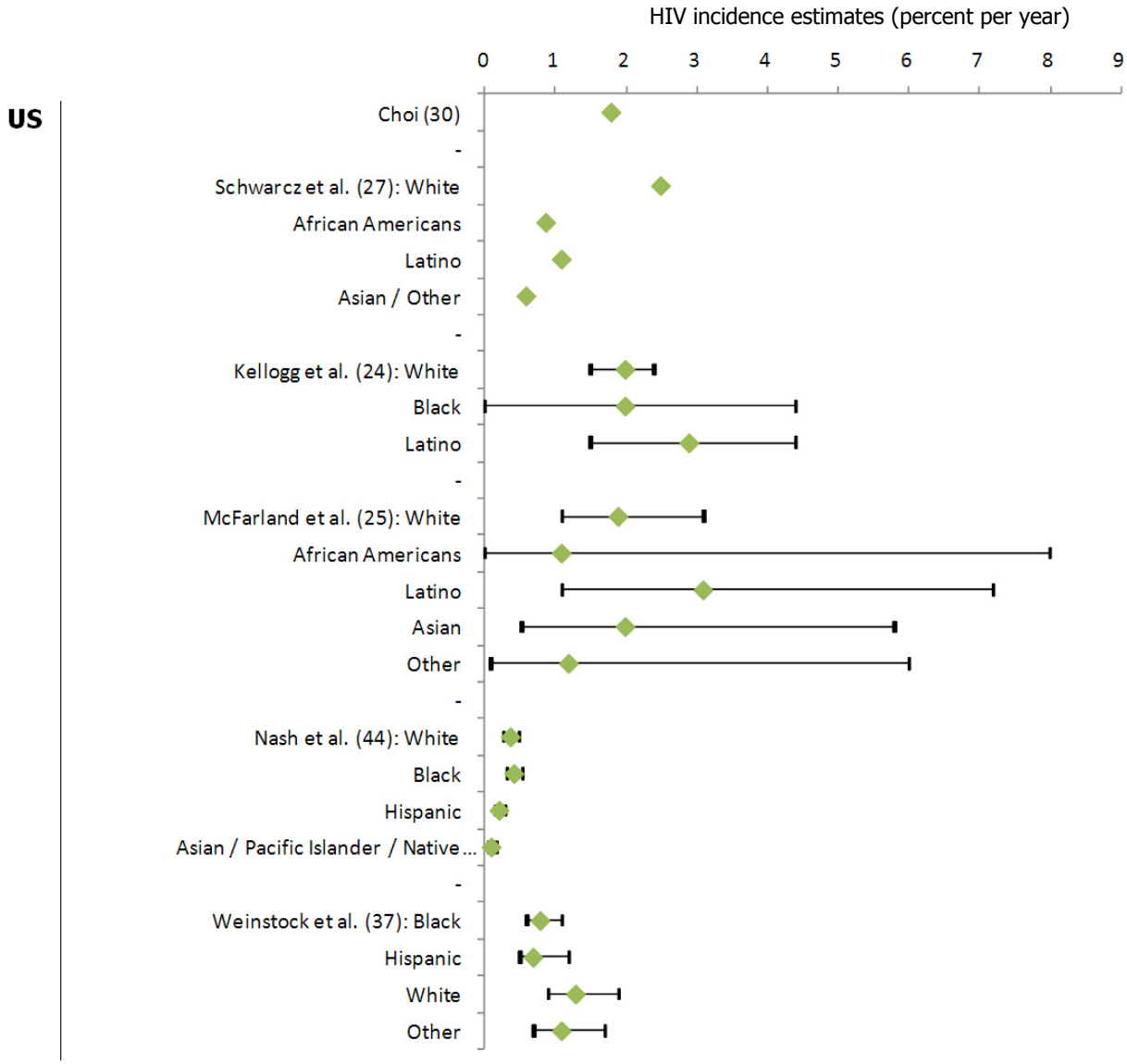
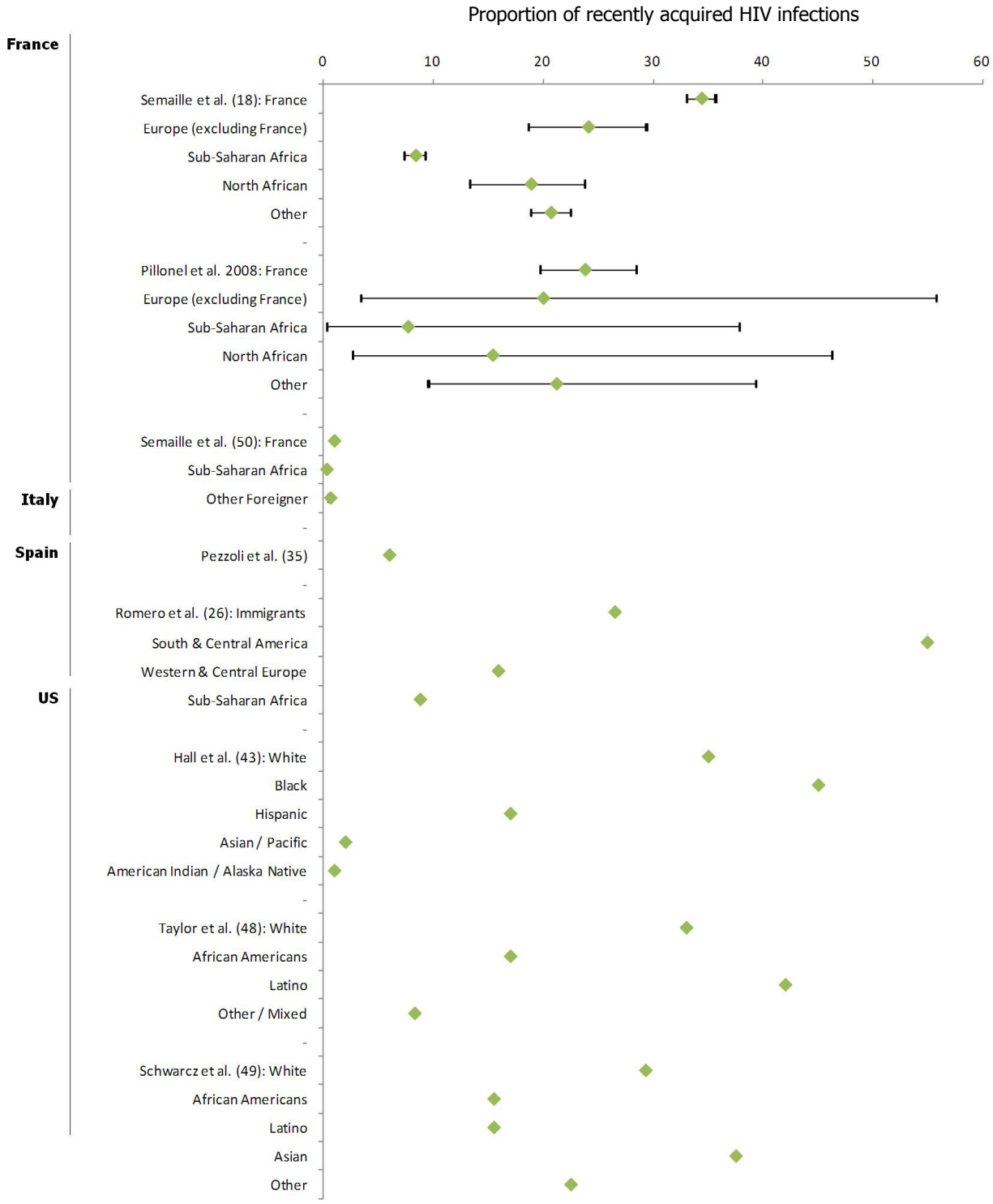


Figure 4-9. Migrants and ethnic minorities



Annex 5: Effect of sample size on confidence intervals

Figure 5-1. Effect of increasing the sample size from 100 to 50 000 on the confidence intervals of an estimated HIV incidence of 1%

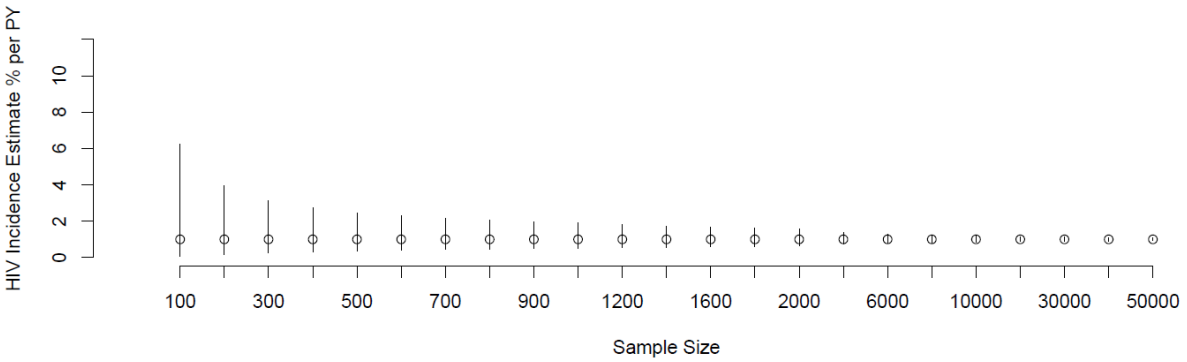


Figure 5-2. Effect of increasing the sample size from 100 to 50 000 on the confidence intervals of an estimated HIV incidence of 2%

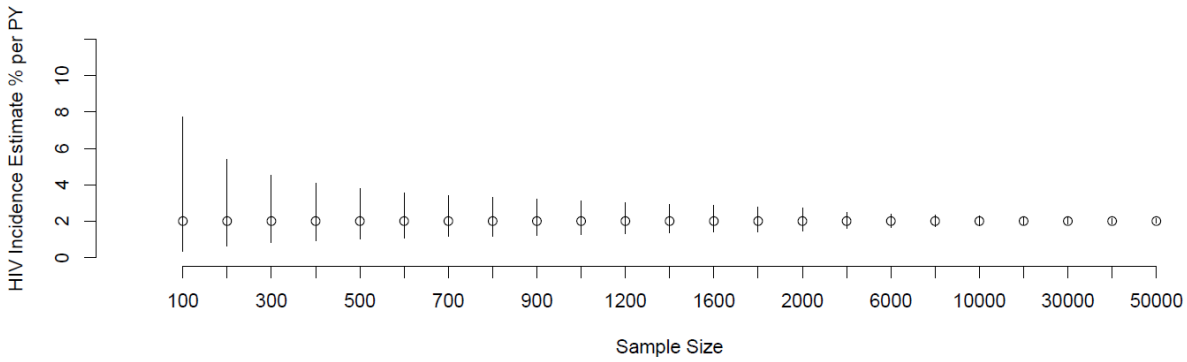


Figure 5-3. Effect of increasing the sample size from 100 to 50 000 on the confidence intervals of an estimated HIV incidence of 3%

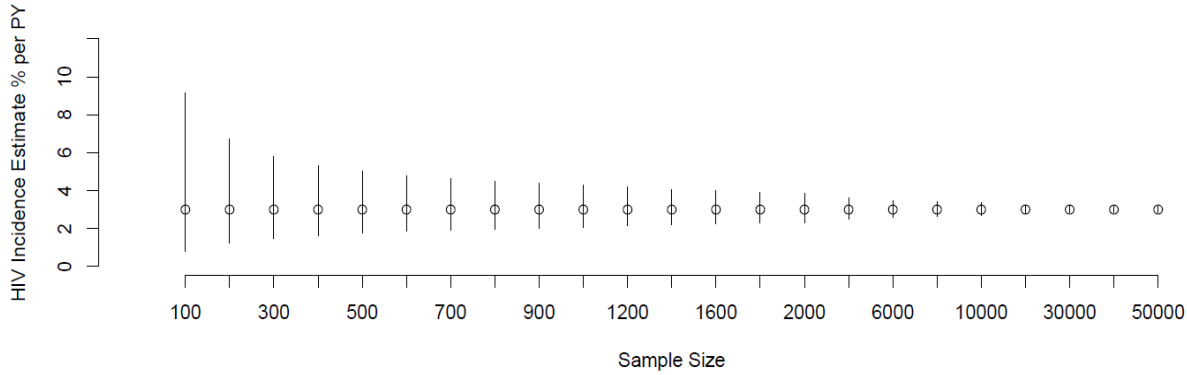


Figure 5-4. Effect of increasing the sample size from 100 to 50 000 on the confidence intervals of an estimated HIV incidence of 4%

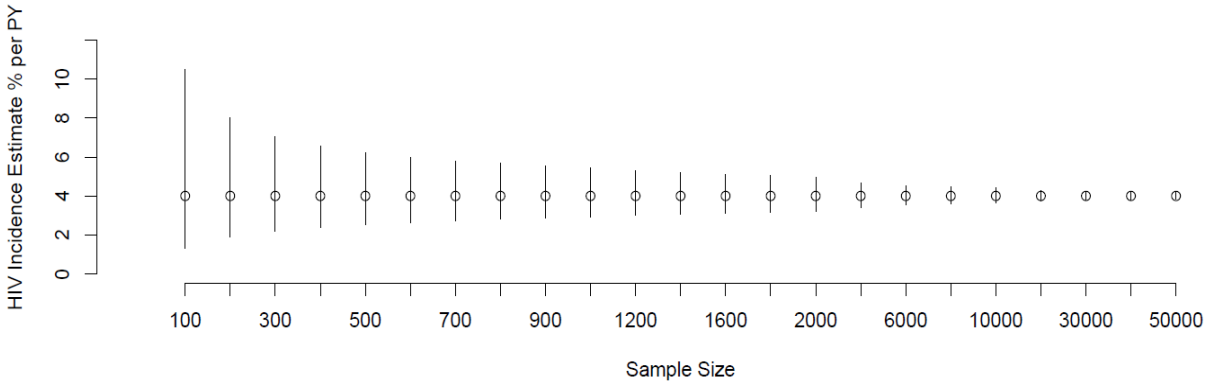
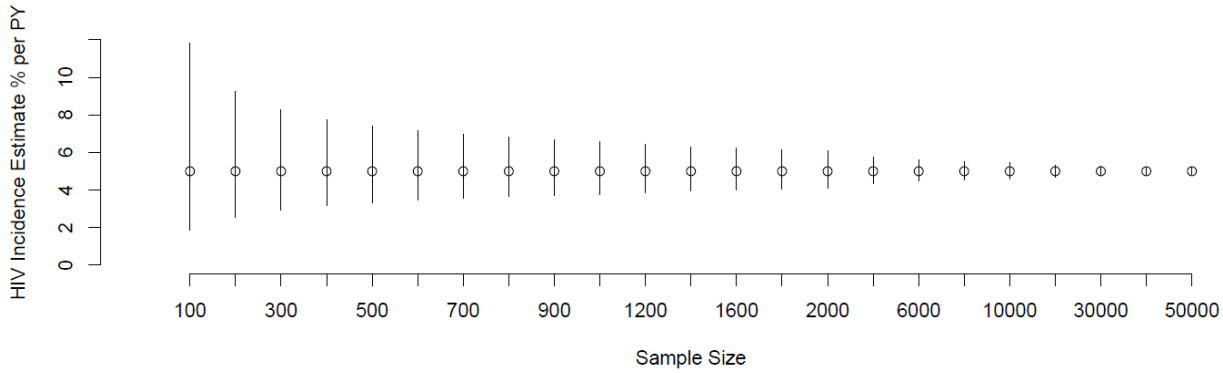


Figure 5-5. Effect of increasing the sample size from 100 to 50 000 on the confidence intervals of an estimated HIV incidence of 5%

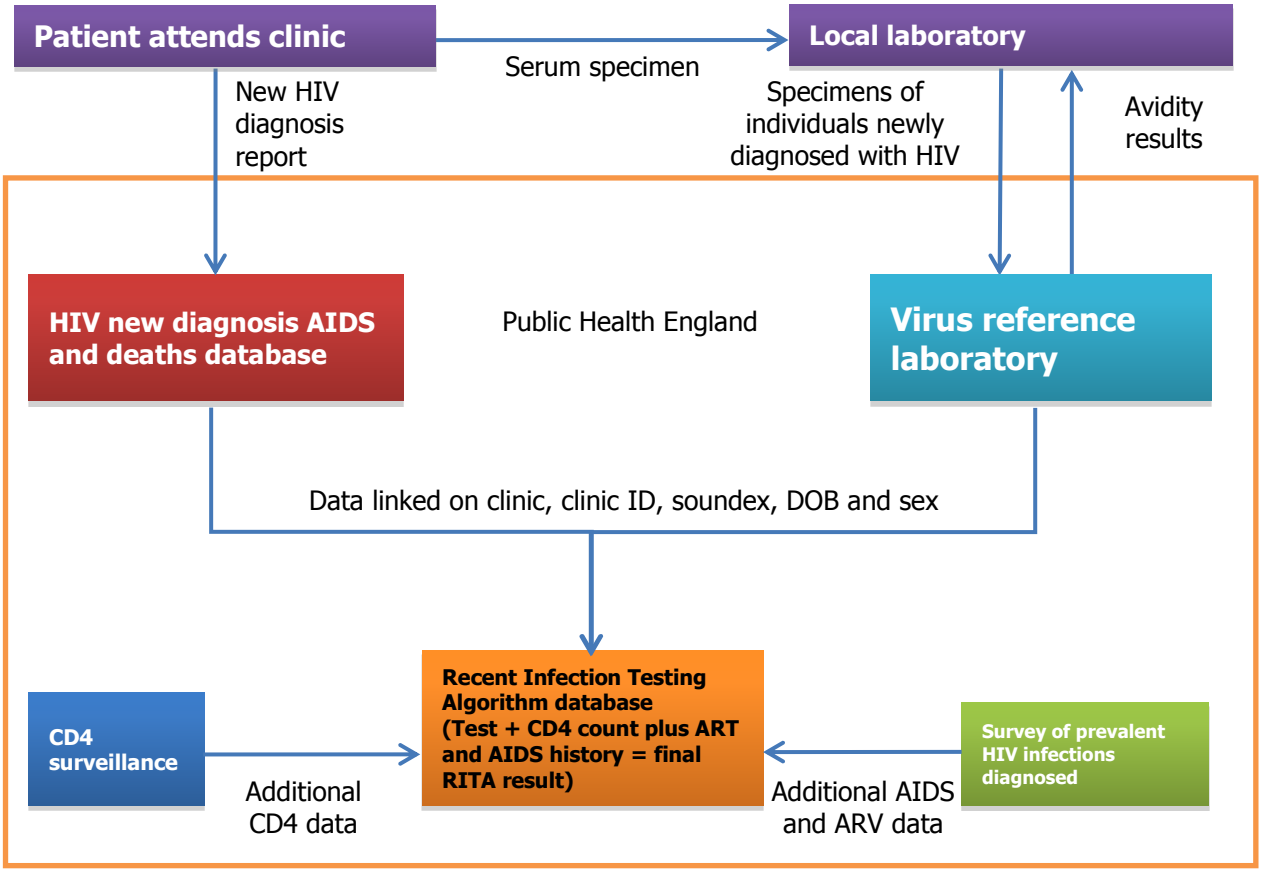


Annex 6: Sample data dictionary for RITA surveillance

Field number	Category/ field name	Description	Coding	Mandatory	Optional
1	Reporting Country	Name of country participating in the pilot	As for TESSy	X	
2	Organisation	Name of communicable disease surveillance organisation	See additional coding sheet	X	
3	Site name	Name of facility	Open ended		X
4	Site type/facility	Type of facility	(e.g. coding example from the UK) • Sexual health clinic • General practice/primary care • Hospital • Drug dependency unit • Prison		X
5	Patient ID	A unique patient identifier assigned by the recruiter	e.g. Soundex, Clinic ID number	X (at country level)	
6	Gender	Patient's gender, specified by the patient	As for TESSy	X	
7	Date of birth	Patient's date of birth	As for TESSy	X	
8	Ethnicity	Patient's ethnicity, specified by the patient	(e.g. coding example from the UK) • White • Black (Caribbean) • Black (African) • Black (other) • Indian/Pakistani/Bangladeshi • Other/mixed • Other Asian/Oriental • Not known		X
9	Country of birth	Patient's country of birth	As for TESSy		X
10	Date of attendance	Date patient was seen as the facility	dd-mm-yyyy		X
11	HIV diagnosis		As for TESSy	X	
12	HIV subtype		As for TESSy	X	
13	Date of HIV diagnosis	Date the patient was first diagnosed with HIV	As for TESSy	X	
	Type of HIV diagnostic test	Type of assay used to diagnose HIV, e.g. western blot.	Open ended		
14	CD4 count	The patient's most recent CD4 count, ideally taken at the same visit as the sample for the test of recent infection.	As for TESSy	X	
15	CD4 count date	The date the most recent CD4 count was taken	dd-mm-yyyy	X	
18	Viral load	The patient's viral load count, ideally taken at the same visit as the sample for the test of recent infection.	Number (copies/ml)		X
19	Date of viral load measure	The date the most recent viral load count was taken	dd-mm-yyyy		X
20	Date of last (negative) HIV test	The date of the last time the patient took an HIV test and has a negative result. Could be laboratory data or patient reported.	dd-mm-yyyy	X	

Field number	Category/ field name	Description	Coding	Mandatory	Optional
21	Number of HIV test in the last two years	The number of HIV tests the patients has had in the two years prior to the visit date	Number		X
22	Transmission	Type of high-risk exposure, e.g. MSM	As for TESSy	X	
23	Country of infection	Country where patient was likely to have been infected	As for TESSy		X
24	ART treatment	If the patient had a recent history if ART, i.e. in the three months before the samples were taken	As for TESSy	X	
25	Start date of ARV use	Date the patient started current ART	dd-mm-yyyy	X	
26	Type of ART treatment	Specific regimen of antiretroviral drugs the patient has been prescribed	Additional sheet for coding will be developed		X
27	Stage	The presence of symptoms of seroconversion	As for TESSy	X	
28	AIDS indicator disease		As for TESSy	X	
29	Co-morbidities/other physical conditions	Any other illness of the patient or physical condition which could affect the result of the assay	• Immunosuppressant types, lupus • Cancer/chemotherapy? • Pregnant • Other		X
30	Motivation for visit	To be based on the patient's response	• Regular tester • Recent high risk behaviour (e.g. UAI, IDU, HIV positive partner) • Symptoms of seroconversion/ primary HIV infection • Referral by a physician • Symptoms of a sexually transmitted infection • Pre-op • Work requirement • Other		X
31	Type of sample	Type of sample taken from the patient for test of recent infection	Dry blood spot Serum	X	
32	Date of specimen	Date the specimen was taken from the patient for test of recent infection (if different from date of recruitment/attendance)	dd-mm-yyyy	X	
33	Type of assay used for test of recent infection	The type of assay used by the laboratory for the test of recent infection	• AxSYM avidity • Architect avidity • BED - CEIA	X	
34	Assay result	The result of the test of recent infection assay	Number	X	

Annex 7: Sample data flow schematic for RITA surveillance



Annex 8: Sample data tables

		15–24	25–34	35–49	50+	All
MSM	Recent infections	39	100	69	13	221
	All diagnoses	125	320	377	99	921
	%	31%	31%	18%	13%	24%
	95% CI	23.2–40.1	26.2–36.6	14.5–22.6	7.2–21.4	21.3–26.9
Heterosexual men	Recent infections	1	14	15	4	34
	All diagnoses	16	97	198	75	386
	%	6%	14%	8%	5%	9%
	95% CI	0.2–30.2	8.1–23.0	4.3–12.2	1.5–13.1	6.2–12.1
Heterosexual women	Recent infections	9	18	14	4	45
	All diagnoses	66	225	261	90	642
	%	14%	8%	5%	4%	7%
	95% CI	6.4–24.3	4.8–12.3	3.0–8.8	1.2–11.0	5.2–9.3
All heterosexuals	Recent infections	10	32	29	8	79
	All diagnoses	82	322	459	165	1028
	%	12%	10%	6%	5%	8%
	95% CI	6.0–21.3	6.9–13.7	4.3–8.9	2.1–9.3	6.1–9.5
All	Recent infections	54	143	106	24	327
	All diagnoses	243	741	963	311	2258
	%	22%	19%	11%	8%	14%
	95% CI	17.2–28.0	16.5–22.3	9.1–13.2	5.0–11.3	13.1–16.0

** Overall, 36% of new HIV diagnoses had a test for recent infection; results were similar across exposure groups.*