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HIV Forensics II

Estimating the likelihood of recent HIV infection

Implications for criminal prosecution





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About this briefing paper

This briefing paper considers the validity and meaning of scientific tests to estimate the likelihood of a recent infection in persons already diagnosed as HIV positive – known generically as RITA tests (Recent Infection Testing Algorithm) – in the context of prosecutions for HIV transmission.

It should be read in conjunction with the original briefing paper on 'HIV Forensics' (NAT/NAM, 2007) which focused on the limitations of another scientific test, phylogenetic analysis – which examines how two or more strains of HIV are related – when used as evidence in the context of prosecutions for HIV transmission.

The information contained within is aimed primarily at professionals working in the criminal justice system and those working in the field of HIV who may be called as expert witnesses in criminal HIV transmission cases. It may also be useful for people working in HIV support organisations as well as persons living with HIV, especially those who are newly diagnosed and/or those potentially involved in criminal HIV transmission cases.

Although this briefing paper is written within the context of the legal and policy situation of England and Wales, it will be of relevance to other jurisdictions, where prosecutions occur for HIV transmission and where tests take place to estimate the likelihood of recency of infection in newly diagnosed individuals.

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Summary and Recommendations

No current scientific test to estimate the likelihood of recent HIV infection is able to conclusively state when an individual acquired HIV.

Those for whom the RITA test suggests that they may have been recently infected may, as a result, believe that they are confident they know who was responsible for infecting them. However, because of the considerable uncertainty around RITA tests on an individual level, this test alone is not an adequate basis for such assumptions.

Conversely, should a RITA test result appear to suggest that an individual was not recently infected, in contrast to other evidence suggesting recent infection, the RITA test result alone would not conclusively exclude the possibility that a recent sexual partner was the source of their infection.

All clinics, if they deliver RITA test results to patients, must communicate clearly and effectively the limitations of such testing and ensure that healthcare providers adhere to a clear protocol for doing so. This should include a requirement that individuals are not only informed verbally of the results of the RITA test, but are also informed of the limitations of the test and given a patient information leaflet.

When considering the possibility that an individual's most recent (or most recent known HIV positive) sexual partner was HIV positive and had not informed the individual, RITA test results should be interpreted in the context of the totality of other evidence and should never be the starting or central point of a criminal investigation.

In addition to the limitations of RITA tests when applied to the individual – as well as CD4 counts and viral loads in establishing that an infection is, indeed, recent, there is evidence that a patient's assumption as to the source of their infection is often inaccurate.

RITA tests are not reliable as indications of recency of infection for individuals in the context of criminal proceedings because:

- they are designed to estimate recency and calculate incidence rates at the population, not individual, level.
- the immune responses of individuals (which are measured in RITA tests) vary but the RITA test for recency corresponds to an 'average' response, hence its usefulness at the population level and its unreliability at the individual level.
- significant rates of false recent results have been repeatedly documented in individuals, i.e. recent infection has been suggested by a positive RITA test, but other means/methods have then demonstrated that the RITA test result was wrong.

For all these reasons, RITA test results can only provide an approximation of the likelihood of recent HIV infection. In addition, this likelihood is difficult to quantify with the scientific certainty required by a court.

Expert witnesses should state unequivocally that RITA test results cannot prove timing of infection in an individual. Such results must therefore be interpreted with caution and only used in the context of all the available evidence, including phylogenetic analysis, CD4 count, HIV testing history, and sexual history.

Introduction

The availability of scientific tests to estimate the likelihood of a recent HIV infection – and the way the results of these tests are communicated and understood - raises important issues of evidence in alleged cases of criminal HIV transmission.

To our knowledge, results of tests to estimate recent HIV infection have not yet been used in the courts to attempt to prove timing of HIV transmission (and from that timing, identity of the person who transmitted HIV to

the complainant). Nevertheless, it is important that high quality information about such tests is made available to all stakeholders potentially involved in criminal prosecutions in order to ensure that such evidence is only used when appropriate and that its limitations are fully acknowledged.

This paper will first examine the use and reliability of tests to estimate recent HIV infection when used as epidemiological tools – i.e. in order to examine the dynamics of the HIV epidemic. It will then highlight the difficulties with interpreting these tests on an individual basis and conclude with recommendations for various stakeholders on the appropriate interpretation and use of these tests in the context of allegations of criminal liability for HIV transmission.

The 'take-home' message of this briefing paper is that no current scientific test to estimate recent HIV infection is able to conclusively state when an individual acquired HIV. Test results, therefore, must be interpreted with great caution when used as evidence in a court of law.

Educating (us about) RITA

Until recently, the generic term used to describe tests for recent infection was STARHS (Serological Testing Algorithm for Recent HIV Seroconversion). In 2009, an international working group convened by the World Health Organisation (WHO) recommended the term RITA (Recent Infection Testing Algorithm). This name change took place because some of the newer tests are no longer *serological* (i.e. do not test serum for antibodies).

There are about half a dozen different tests used in algorithms (protocols) to estimate recent HIV infection. Types of test include:

- detuned;
- ▶ BED-EIA: and
- avidity.

A RITA test will use one or more of these tests in combination with other clinical data known to potentially affect the results of test(s) being used. For example, this may require identifying individuals with low antibody titres (concentrations) due to:

- anti-retroviral therapy (ART);
- advanced HIV disease (i.e. AIDS);
- unusual immune responses; or
- certain medical conditions including hyperor *hypo-gammaglobulinemia* (unusually high or low concentrations of antibody protein in *blood plasma*) and/or pregnancy.

How tests estimate the likelihood of recent HIV infection

Tests to estimate the likelihood of recent HIV infection are based on the principle that immune responses to HIV develop over time (see 'How the immune system fights HIV').

The test used for RITA testing in the United Kingdom is known as an avidity test. This measures the overall strength of the bond between an antibody and antigen – the stronger the bond the longer the person is likely to have been infected. Typically, people with a weaker bond will have acquired HIV more recently than people with stronger bonds.

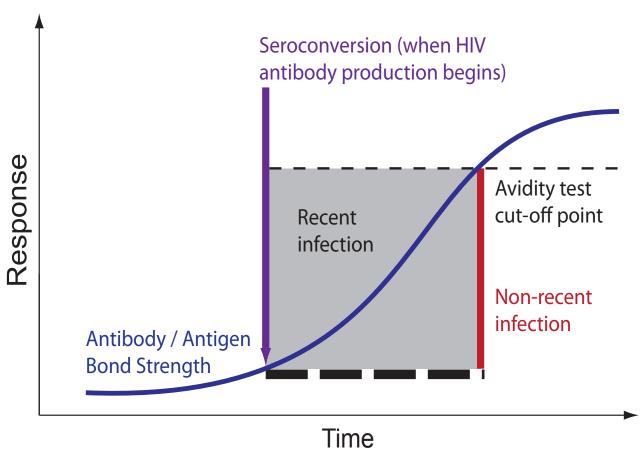


Figure 1: How avidity tests can estimate the likelihood of recent infection, adapted from Mastro TD et al.

For public health monitoring purposes, a cut-off point is chosen (based on a population average) which best separates people most likely to have recently-acquired their infection from those who appear to have longer-term infection. The cut-off point for the avidity test is around 142 days (4.7 months) after infection.

Other RITA tests that measure different qualities of the antibody response – such as the antibody concentration or proportion - have different cut-off points resulting in different definitions of 'recent' infection. Depending on the test used, these range between one and six months after infection."

How the immune system fights HIV

Soon after an individual acquires HIV, their immune system begins to produce HIV antibodies. Antibodies are proteins produced by the immune system in response to foreign bodies (such as viruses, bacteria or pollen) that appear to be a threat. The immune system recognises characteristic proteins on these foreign bodies called antigens and responds by generating corresponding (The word 'antigen' derives from combining two words, antibody generation.) Antibodies to HIV usually begin to appear in the blood at low concentrations within one or two weeks of infection. HIV antibody concentrations continue to increase for several more months, usually reaching a peak approximately six months following infection, and remain at these levels indefinitely unless the immune system is severely weakened by advanced HIV disease (AIDS). However, an individual's immune system can respond differently to what is typical, and some people are 'rapid responders' (i.e. producing antibodies earlier than the average) and others are 'slow responders' (i.e. producing antibodies later than the average). In addition to this, about 2% to 5% of people living with HIV have a natural ability to partially control HIV infection and are known as "long-term non-progressors", and less than 1% of the population have a natural ability to completely control HIV infection, and are known as "elite controllers". This also has an impact on the number and quality of HIV antibodies measured by RITA tests. Since individual immune responses can vary so widely, tests to estimate recent infection that rely on comparisons with a typical response after a certain period of time, can never be conclusive on an individual level. For more on the individual limitations of RITA tests, see 'Why these tests are hard to interpret accurately for individuals'.

RITA tests for public health use

A current limitation of the HIV antibody test – various versions of which are used globally to diagnose HIV infection^{iv} – is the fact that no currently available HIV antibody test can reveal when an individual acquired HIV.

This limitation is especially important to scientists, policy-makers and funders who require details not only of the number of people living with HIV (known as HIV prevalence), but also the rate at which people are being newly infected (known as HIV incidence).

Estimating the number of people recently infected can provide a guide to HIV incidence. This can then be used to plan, fund and evaluate the impact of HIV prevention services and policies.

In 1998, the first scientific tests to estimate the likelihood of recent HIV infection were introduced as research and epidemiological tools in the United States. Since then, other tests to estimate the likelihood of recent infection have been developed to better estimate the rate at which people are being newly infected. A number of RITA tests and techniques have been explored and there are currently about a half a dozen tests used in population studies and for surveillance purposes.vi Very few laboratories in any given country have the expertise to do such testing.

The UK is the only country in the world at present to return individual RITA test results to patients. Other countries, including France and the United States, routinely test the blood samples of newly diagnosed patients to estimate the likelihood of recent infection for public health use, but do not return these results to patients.

Understanding the limitations of RITA tests

The properties and reliability of RITA tests vary across populations and their accuracy has not been fully studied to date. A 2009 WHO report^{vii} notes that the development of standardised tests to estimate the likelihood of recent infection faces several important challenges including:

- impact of anti-retroviral therapy (ART) and advanced HIV disease (AIDS) on test accuracy;
- difficulty in standardising cut-off points across different platforms;
- difficulty with calibrating tests and other quality control issues; and
- complexity and high cost of some of the tests.viii

Although the various tests used to estimate the likelihood of recent HIV infection have been validated and the latest versions of these tests perform well on a population level, RITA test results can only provide an approximation of the likelihood of recent HIV infection.

These limitations are acknowledged by global experts in the field^{ix} and currently no test used for RITA is endorsed by national^x or international normative agencies (i.e. the Joint United Nations Programme on HIV/AIDS (UNAIDS)xi or the WHOxii) for individual diagnostic use.

The WHO is working with various stakeholders to validate and better define the performance of RITA tests.



xii WHO. Development of assays to estimate HIV incidence: meeting proceedings, Chapel Hill, North Carolina, May 13 - 14, 2009.

WHO. Development of assays to estimate HIV incidence: meeting proceedings, Chapel Hill, North Carolina, May 13 - 14, 2009.

viii Ihid

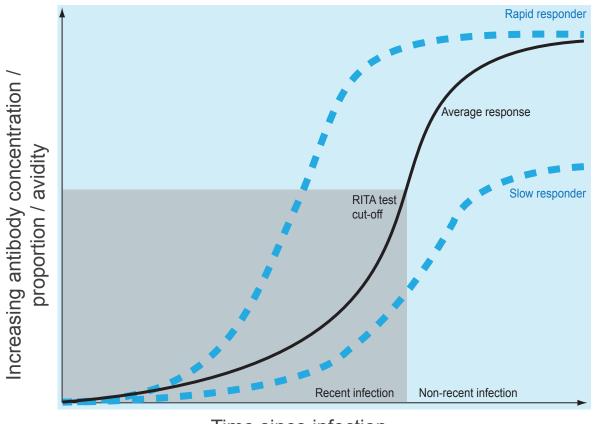
[™]WHO. WHO Technical Working Group on HIV Incidence Assays: Meeting Report, Cape Town, South Africa, 16 and 17 July 2009.

^{*}US Centers for Disease Control and Prevention. CDC HIV/AIDS Science Facts: Using the BED HIV-1 Capture EIA Assay to Estimate Incidence Using STARHS in the Context of Surveillance in the United States. October 2007

i UNAIDS. Statement on the use of the BEDassay for the estimation of HIV-1 incidence for surveillance or epidemic monitoring. Meeting of the UNAIDS/WHO Reference Group on Estimates MaP. Athens, Greece; 2005

Why RITA test results are hard to interpret accurately for individuals

In addition to the limitations listed above, due to individual variability in producing HIV antibody responses (see 'How the immune system fights HIV'), any current test is likely to lead to some additional misclassifications when examining individual samples. This is because the test's cut-off point is based on the average time a group of individuals will develop peak antibody concentrations.xiii



Time since infection

Figure 2: Variations in individual responses to RITA assays, adapted from Murphy G, Parry JV.xiv

By definition there will always be individuals who do not have the average antibody response. Consequently, RITA result misclassifications at the individual level are not uncommon, with documented false-positive, i.e. falserecent rates - the percentage of persons who were labelled recent who should have been reported as non-recent estimated as between 2% to 15%.xv

This matters less on a public health level, because there will usually be an averaging out of rapid and slow responders. However, on an individual level, even if other information known to affect the test is factored in (for example, CD4 count and ART use), there remains a real possibility that any individual test result will be a misclassification.

Delivering RITA test results to individuals

To our knowledge, the United Kingdom is the only country in the world currently returning RITA results to newly diagnosed patients as a matter of routine.xvi

It is important that healthcare providers delivering this information to newly diagnosed individuals understand the limitations of such testing and communicate this clearly and effectively to their patient.

Before doing so, the RITA test result should be considered in the context of other medical and clinical information, such as a patient's recall of their recent HIV risk behaviour and the state of their immune system. Even if all of these are consistent with recent infection, the test result can still only suggest a likelihood that a patient's HIV infection was recent.

The Health Protection Agency (HPA) has produced a patient information leafletxvii which states the following:



The [RITA] test will give us an indication as to whether or not you are likely to have been infected within the last 6 months. It is important to understand that the [RITA] test results must be interpreted with caution as it gives only an approximate indication.

All clinics, if they deliver RITA test results to patients, must ensure they have a clear protocol for healthcare providers to follow in doing so. This should include a requirement that individuals are not only informed verbally of the RITA test results and of the test's limitations, but are also given the HPA leaflet, or a similarly worded locally-produced patient information leaflet.

RITA non-progressors or regressors?

WHOxviii defines individuals who are misclassified in RITA tests in two different ways. RITA non-progressors are individuals that stay RITA-recent because they never develop immune responses that would take them past the cut-off point of the RITA test. On the other hand, RITA regressors are individuals that have typical immune responses early on, but then have a weakened immune system which would show up on a RITA test as 'recent' despite having been infected some years ago. In both instances someone will appear as recently infected even though in fact they were infected some time ago.

Implications for criminal investigationsxix

Proving criminal HIV transmission requires the use of a combination of scientific evidence, medical records and testimony in order to attempt to reconstruct the fact, timing and direction (i.e. who infected whom) of the HIV transmission event under investigation.

It may be difficult to prove any of the three beyond reasonable doubt, particularly if the complainant has never previously taken an HIV test, because there may be plausible alternative explanations for how HIV infection could have occurred.

RITA test results may appear to be relevant to the question of the timing of the alleged infection. This will then be relevant to the consideration of who might have been the person to have passed HIV on to the complainant. It will also be relevant to questions around the knowledge that both any person investigated and the complainant had at the probable time of transmission (and knowledge relates to criminal liability), in particular:

- Did the alleged HIV transmission take place before or after the person being investigated was made aware of his or her HIV status? (In most jurisdictions, including England and Wales, criminal liability only falls on persons aware they are, in fact, HIV positive.)
- Did the alleged HIV transmission take place before or after the complainant was made aware of the HIV status of the person being investigated? (In some jurisdictions, including England and Wales, the complainant's awareness of the risk of HIV transmission would eliminate criminal liability for the defendant.)

Those for whom the RITA test suggests that they may have been recently infected may, as a result, believe confidently they know who was responsible for infecting them. However, because of the considerable uncertainty around the validity of RITA tests on an individual level, this test alone is not an adequate basis for such assumptions. Conversely,

should a RITA test result appear to suggest that they were not recently infected, in contrast to other evidence suggesting recent infection, a RITA test result alone would not conclusively exclude the possibility that a recent sexual partner was the source of their infection.

Other markers - CD4 count/viral load

Attempting to work out the timing of HIV infection via the impact of HIV upon the immune system (as measured by absolute numbers of key immune cells targeted by HIV, known as CD4 cells) or viral load levels at an individual level is as problematic as interpreting RITA test results.

This is also due to the wide individual variability in both viral load and CD4 count at any point – from initial infection onwards. Although CD4 counts and viral loads tend to be higher earlier in infection than in later infection, there are many individual exceptions to this, making it extremely difficult to draw firm conclusions regarding the length of time the virus has been reproducing in a person's body.xx

"During 2009, 1,741 individuals were tested using the Recent Infection Testing Algorithm (RITA) as part of the national monitoring of recent HIV infections in England and Wales. In total, 1 in 10 (196/1,741) HIV infections were classified as probably acquired within the previous 4-5 months, including 1 in 6 (17%; 123/745) in MSM and 1 in 16 (6.9%; 54/783) in heterosexuals. Among MSM, similar proportions of recently acquired infections were seen across all age groups from 18% (20/110) in those aged 15-24 to 14% (10/73) in those aged 50 and over. Among heterosexuals, however, the highest proportion of recent infections were in those aged between 15-24 years (16%; 12/73) and 25-34 years (12%; 9/73) for females and males, respectively." HIV in the United Kingdom: 2010 Report. HPA 2010

Limitations of patient recall

In addition to the serious limitations of RITA tests, CD4 counts, or viral loads in establishing that an infection is indeed recent, there is evidence that a patient's assumption as to the person most likely to be the source of their infection - often their most recent sexual partner - may be inaccurate.

A study in Cuba found that around two-thirds of heterosexuals who named their most recent sexual partner as the likely source of their infection during routine contact tracing appeared to be mistaken because phylogenetic analysis suggested that this partner was unlikely to have been the source of their infection.xxi Similarly, a study of gay men in California who had been very recently infected found that a third were mistaken when they assumed that their most recent sexual partner was the most likely source of their infection because phylogenetic analysis suggested that this partner was unlikely to have been the source.xxii

Allowing for the possibility of other sources of infection

Someone who has recently been diagnosed HIV positive and has received a RITA-recent test result may still be mistaken regarding the source of their infection. Just because they have discovered that their most recent sexual partner is HIV positive and had not informed them, does not necessarily mean that they acquired their own recently diagnosed infection from that partner.

Complainants in criminal HIV transmission investigations might not have undergone HIV testing until after ending the relationship with the accused. However, unless medical history suggests no other



possible prior HIV risks - sexually or otherwise - it would be wrong to assume that a complainant was HIV negative prior to his or her relationship with the defendant, in the absence of a documented, previous negative HIV antibody test.

Even when phylogenetic analysis suggests that the viruses in both people are very closely related in comparison with other samples, this does not eliminate the possibility that a third (or fourth) party may have infected the complainant.

Studies have found that people can share similar viruses with many other people who are part of a wider transmission network (for example, individuals who have current or former sex partners in common, whether they know it or not, and the sex partners of those partners).xxiv This means that there are multiple ways to account for why a complainant and defendant may have highly similar strains of HIV. For example, the defendant could have unknowingly transmitted HIV to an intermediary who then infected the complainant, or both the defendant and complainant could have acquired very similar strains of HIV from a third party.

xxi Resik S, Lemey P, Ping LH, et al. Limitations to contact tracing and phylogenetic analysis in establishing HIV type 1 transmission networks in Cuba. AIDS Research and Human Retroviruses 2007 Mar; 23(3):347-56.

xxiii For a full discussion of the limitations and use of phylogenetic analysis as evidence in criminal investigations of HIV transmission, see: NAT/ NAM. HIV Forensics, 2007. Available at: http:// www.nat.org.uk/Media%20library/Files/PDF%20 Documents/HIV-Forensics.pdf

xxiv Brenner BG et al. High rates of forward transmission events after acute/early HIV-1 infection. J Infect Dis 195: 951-59, 2007.

Recommendations for expert witnesses

In criminal HIV transmission cases the expert opinion of medical experts is of critical importance. They may be allowed to express an opinion on whether the scientific and medical evidence is sufficiently persuasive to indicate that the defendant was the only possible source of the complainant's infection or not.

Consequently, expert witnesses must be clear about the limitations of RITA tests and over-interpreting such test results is unacceptable.

RITA tests are not conclusive as indications of recency of infection for individuals in the context of criminal proceedings because:

They are designed to estimate recency and calculate incidence rates at the population, not individual. level.

The immune responses of individuals (which are measured in RITA tests) vary but the RITA test for recency corresponds to an 'average' response, hence its usefulness at the population level and its unreliability at the individual level.

Significant rates of false recent results have been repeatedly documented in individuals. i.e. recent infection has been suggested by a positive RITA test, but other means/methods have then demonstrated that the RITA test result was wrong.

Experts should therefore state unequivocally that RITA test results cannot prove timing of infection. Such results must be interpreted with caution and only used in the context of all the available evidence including phylogenetic analysis, CD4 count, HIV testing history, and sexual history.



Glossary

AIDS (Acquired Immune Deficiency Syndrome) the most advanced stage of HIV infection. It describes a collection of specific illnesses and conditions which occur because the body's immune system has been damaged by HIV. Thanks to anti-retroviral therapy, it is now possible to be infected with HIV and either never experience AIDS or to recover from an AIDS-defining illness.

algorithm

a specific set of sequential instructions for carrying out a procedure, also known as a protocol.

antibody

a protein substance produced by the immune system in response to a foreign organism.

antigen

something the immune system can recognise as 'foreign' and attack.

anti-retroviral therapy

a combination of usually three individual drugs that act against retroviruses such as HIV, often abbreviated to ART or ARV drugs (short for anti-retroviral drugs).

avidity

the overall strength of the bond between an antibody and antigen.

BED-EIA

the only commercially available HIV incidence test, measuring the proportion of HIV-specific antibodies that appear against HIV subtypes B, E, and D (hence the name 'BED') as measured by a capture enzyme immunoassay (EIA). The test is now known to be particularly unreliable and national and international agencies now recommend against using it.

blood plasma

the fluid portion of the blood.

detuned

an early RITA test that is no longer in common use. A detuned test is a much less sensitive HIV antibody test than is regularly used to detect HIV infection. A negative detuned test combined with a positive antibody HIV test can suggest recent infection.

epidemiological

relating to the study of disease within a population.

HIV (Human Immunodeficiency Virus)

the virus that, if untreated, can lead to AIDS.

HIV incidence

the rate at which people are being newly infected within a particular population or geographic region.

HIV prevalence

the total number of people living with HIV within a particular population or geographic region at a specific point in time.

hyper-gammaglobulinemia

unusually high levels of specific antibody proteins in blood plasma.

hypo-gammaglobulinemia

unusually low levels of specific antibody proteins in blood plasma.

recent HIV infection

a relative term, depending on which RITA test is being used. It is, however, commonly used to mean infection within the previous six months.

serological

tests relating to antibodies contained within blood serum.

STARHS (Serological Testing Algorithm for Recent HIV Seroconversion)

the former name for RITA tests, and may still commonly be used to describe these tests.

titre

a laboratory measurement of the amount, or concentration, of a given component in solution.

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About NAT

NAT is the UK's leading charity dedicated to transforming society's response to HIV. We provide fresh thinking, expertise and practical resources. We champion the rights of people living with HIV and campaign for change.

SHAPING ATTITUDES.
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All NAT's work is focused on achieving four strategic goals:

- Effective HIV prevention in order to halt the spread of HIV
- Early diagnosis of HIV through ethical, accessible and appropriate testing
- Equitable access to treatment, care and support for people living with HIV
- Eradication of HIV-related stigma and discrimination.

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