Appendices

HIV testing and risks of sexual transmission

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Watch for out-of-date information

Appendices

Three appendices for the i-Base guide to HIV testing and risks of sexual transmission include more technical details about HIV testing.

Print copies of this booklet do not include these sections which are all available online:

http:///www.i-Base.info

The online versions include web pages and PDF formats for these additional 14 pages.

Appendix 1: Different types of HIV test

This section explains in detail the difference between the main types of tests used to test for HIV and when they are used.

These are:

- Antigen only (p24 tests). These are rarely used.
- Antibody only tests (Ab). These are rarely used because of more recent availablility of joint Ag/Ab tests.
- Combined antibody-antigen tests. These are the most commonly recommended tests in the UK. these test for both antibodies to HIV and p24.
- Viral load tests (RNA PCR test)

  Viral load tests are not approved to diagnose HIV but are sometimes used in some circumstances.

Appendix 2: Theoretical risk, population risk & individual risk

This section discuss the differences between individual risk and population risk. Sometimes what is a very small individual risk may still not be acceptable for many people.

It also includes a brief section about how difficult it is to judge risks and how we approach the idea of risk in daily life.

Appendix 3: How HIV tests work

This section decribes in more detail about how HIV test work.

Sometimes i-Base gets asked technical details and so these might be useful for some people.

It talks about antigens and anitbodies and explains how each of these tests work.

It also explains the differences between Elisa and western blot tests.

This section also includes more detailed information and the timing of different stages of early infection and seroconversion.

If you do not have access to the internet please contact i-Base and we can post you a print out of these sections.

Appendix 1: Different types of HIV test

Different HIV tests work by looking for different things.

1) Proteins on the surface of the virus (antigens/Ag) like protein 24 (called p24).
2) An immune response to the virus (antibodies/Ab).
3) Genetic material from the virus (HIV RNA or DNA).

In this section we describe the main tests.

These are:

- Antigen only (p24 tests). These are rarely used.
- Antibody only tests (Ab). These are rarely used because of more recent availablilty of joint Ag/Ab tests.
- Combined antibody-antigen tests. These are the most commonly recommended tests in the UK. these test for p24 plus antibodies.
- Viral load tests (RNA PCR test)

More details about how the tests work and the science behind them is included in Appendix 3.

GLOSSARY: antigen (Ag): a foreign substance that generates an immune response antibody (Ab): a type of immune cell first made when your body recognises an antigen.

Antibody tests

The most common HIV test is an antibody test.

Antibodies are part of your immune system that are produced when you come into contact with an infection. Antibody tests look for this immune response.

These tests can be finger-prick tests or use blood samples sent to a laboratory.

If this result is negative or non-reactive, then you are HIV negative.

If the result is positive this does not mean that you definitely have HIV, although it is likely. A small percentage of people can have a ‘false-positive’ result.

All positive results need to be confirmed by a second test.

In the UK a more sensitive antibody test called a western blot test is usually used to confirm a positive result. The western blot test takes longer (usually a week). It identifies genuine positive results.

HIV antibody tests do not work as soon as you are infected because it usually takes four weeks for your body to generate antibodies to HIV. The time between infection and when your body makes antibodies is called the ‘window period’.

Most people generate an antibody response within 4 weeks, but occasionally it can take longer. This is why people are advised to wait three months to take an HIV test, or to re-test three months after an earlier negative result.

Taking an antibody test less than 4 weeks after exposure will not tell you very much.

Combined antibody/antigen tests

It is now common for antibody tests to also test for antigens. These are called 4th generation tests or combined antibody/antigen (Ag/Ab) tests.

In these tests the antigen being tested is a major HIV protein called p24.

p24 (short for protein 24) is produced 2–3 weeks after infection and before antibodies are produced. p24 levels are only detectable for the next 1–2 months. However, by the time the p24 levels have dropped antibodies will be present.

4th generation (Ag/Ab) tests are recommended four weeks after exposure. They give an earlier result than antibody-only tests that are recommended after six weeks.

4th generation tests detect over 95% of infections at four weeks after exposure.

As with antibody only tests, a small percentage of people (less than 5%) may have a delayed response to HIV. So a negative test at four weeks needs to be confirmed after three months.

Viral load (RNA PCR) test

PCR stands for Polymerase Chain Reaction. This test looks directly for HIV in blood. It has the shortest potential window period and can be used from 3 days to 4 weeks after an exposure.

Viral load tests are not recommended for HIV testing except in specific circumstances. This is because they are less accurate. They are also more expensive and take longer to get a result.

After infection, viral load is usually very high within the first 4 weeks and so this test can be used to confirm a suspected early infection if someone has symptoms.

If symptoms are related to HIV, then the viral load test will be positive. HIV symptoms are related to viral load.

However, some people have undetectable viral load without treatment, so a negative result does not guarantee that you do not have the virus.

In adults, viral load tests are only usually offered when there is both:

i) A recent high risk exposure (ie condom break with a known HIV positive partner who is not on treatment); and

ii) Symptoms of HIV infection (fever, extreme tiredness, heavy 'flu-like illness etc).

PCR testing for HIV DNA is mainly used for babies born to HIV positive mothers.

As a baby has the mother's antibodies for the first 18 months, antibody testing is not used until a child is two years old.

Which test is which?

Figure 12 lists commonly used HIV tests and shows what type of test they are.

Your testing centre should tell you this information for the test that they use.

Sometimes testing centres give the tests explained above different names like 'ELISA' or 'Western blot' without explaining what kind of test they are and what it is they are looking for.

ELI, MEIA, ELFA, ECLIA use similar technology to ELISA tests.

UK guidelines recommend using 4th generation tests but 5% of clinics still use 3rd generation tests.

Ask your clinic for more detailed information about the type of test that they use.

Figure 12 - Different types of HIV test

Type of test	**What the test look for?**		
	RNA/ DNA *	Anti-gen	Anti-body
PCR/viral load	●		
p24 only test (Ag)		●	
4th generation antigen/antibody (Ag/Ab) tests (p24+ ELISA, ELI, MEIA/ ELFA/ECLIA): includes Architect, Duo, Combo/Combi etc		●	●
1st/2nd/3rd generation antigen only tests (ELISA, ELI, MEIA/ELFA/ ECLIA): includes TriDot etc			●
Rapid tests: finger prick and oral swab test are antibody only: includes OraQuick.			●
Western blot tests look for antibodies to specific HIV proteins. They confirm a positive HIV antibody test result.			●
* Viral genetic material			

When can each test be used?

Viral load can sometimes be detected within a week, p24 on average by day 16 and antibodies by day 25. However, these are average results a lot of people take longer.

A test that misses half of infections is not very useful.

So a 4th generation antigen/antibody test is recommend four weeks after exposure because it will detect 95% of infections.

Validating the timing of viral load (RNA), p24 and antibodies is difficult. Tests can only be checked against blood samples from the same people before and after infection. These are usually people who regularly donate blood (usually twice a week).

Some of these people catch HIV without knowing it. When this picked up in blood screening, these samples are used for testing new HIV tests.

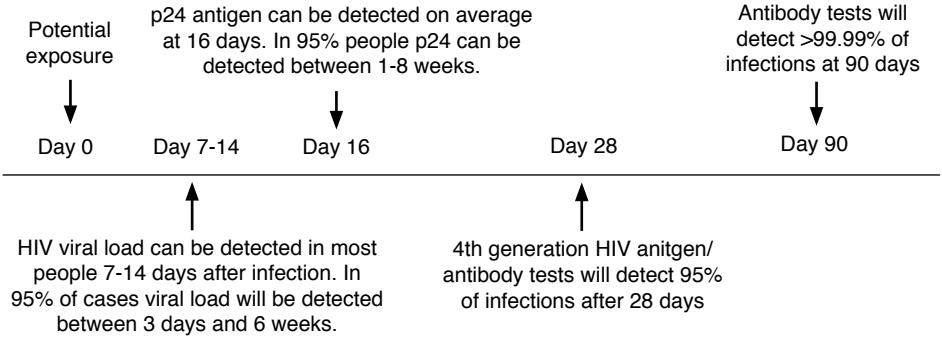
This is why it is impossible to give the percentage chance that a test will be accurate for each day. The tests have been checked on a limited number of samples. These sample reflect the large range of individual responses.

On average, viral load tests (PCR RNA) with a cut-off of 50 copies/mL detect infection about 7 days before a p24 antigen test and 12 days before an antibody test.

These relative times are only used when comparing new tests. They are not good at setting an absolute cut off at 14 days or 19 or 41 days etc.

Figure 13 shows the time ranges after an exposure. Very rarely an antibody response may take longer. Even more rarely (less than 1 in a million) an infection may not make antibodies. These people have positive viral RNA and DNA.

Figure 13 – Average time after exposure to detect HIV antigens and antibodies



Appendix 2: Theoretical risk, population risk & individual risk

Theoretical risk

As HIV can be found all over the body there are instances where in theory there could be a risk of infection but in real life this risk is zero.

The examples on page 21 in the section Ways HIV is NOT transmitted are driven more by an over-active imagination than by real-life risks.

Theoretical risks can never be disproved. It is not possible to prove that something can never happen. So some health information refers to some zero risk activities as low risk.

Estimated population risks?

Guidelines often refer to population risks for different activities. For example, giving oral sex to a man with ejaculation is sometimes referred to as a 0.09% risk (9 in 10,000).

Most of the risk statistics come from population studies that use the following equation to find the percentage risk of transmission:

Risk of transmission =

risk that source is HIV positive

x risk of exposure

This equation should consider both the transmission risk factors discussed earlier and the percentage of people in the population who have HIV.

For example, risks will be higher in a country where 25% of people are HIV positive compared to a country where the less than 1% have HIV.

Population risk vs Individual risk

Population risks are based on many cases where both partners are HIV negative and where the risk is zero.

Using the example above, if out of 10,000 people, nine become positive who only reported oral sex then the population risk (in this population) is 0.09% (9 in 10,000).

But the actual risk for each of those six people at the time they caught HIV was much higher than 0.09%.

Factors including their partner's HIV status, viral load, genetics, STIs, circumcision etc may mean that the risk when the infection occured may have been 10% or 20% (1 in 10 or 1 in 5).

Someone thinking this is only a 0.06% risk would be wrong. If they had been thinking this might be a 20% risk, they may not have become infected.

This is why population estimates should be used cautiously for personal risk.

HIV transmission is often simplified and these important aspects are missed.

Without this detail, prevention advice only tells half the story.

Too often the detail is left out.

Any risk should be put into context of other factors.

This is especially relevant when you may know very little about your partners health.

What is individual risk of HIV transmission?

Individual risk is very difficult to estimate. For some situations the risk could be much higher. For example, if a negative person is giving a positive person oral sex and the following risk factors were included:

- The HIV positive person has a very high viral load of 10 million copies/mL
- The HIV negative person has poor gum health, or recently brushed their teeth, or eaten a packet or crisps that scratched a gum etc
- The positive person receiving oral sex ejaculates in mouth of his partner

The individual risk here could easily be 90% or 50% or 10% or 1%, it is impossible to say which because this level of detail doesn't exist in any of the studies available.

It will definitely be much higher than the risk of 0.09% estimated and referenced in many guidelines on HIV transmission.

Risks in daily life

Finally, there is probably a reason to talk in general about attitudes to risk in daily life.

Some people choose risks in their daily life that others would find impossible. Many jobs have much higher risks than others.

Sometimes people do things after having considered the risks. Often the risk is assumed to be so low that 'it will never happen to me'.

For others, the personal anxiety and worry about all sorts of risks restricts and limits the activities in their lives.

Most people find a balance. Or we like risks in some areas of out lives but not in others.

Flying in a plane or driving a car are all associated with real risks for some people, but the risk is small enough for most people to still travel.

The Canary Islands and San Francisco are popular holiday destinations despite one being an active volcano and the other being on the San Andreas fault line.

Sexual health risks are different in important ways:

- Condoms can reduce your risk to zero.
- HIV usually takes many years to progress.
- HIV treatment dramatically increases life expectancy to one similar to HIV negative people.

Appendix 3: How HIV tests work

What is an antigen?

An antigen is a substance found on a foreign organism such as a virus or bacteria which, when it gets into the body, stimulates an immune response.

What is an antibody?

An antibody is a certain type of immune cell. In adults it is initially made when your body first recognises an antigen.

Antibodies are 'Y' shaped and the two arms of the 'Y' are known as the variable region. This means it is specifically coded to interact with a certain antigen, just as a key is specific for a lock (see Figure 14).

The stick of the 'Y' is the same in all antibodies (the constant region). It is the variable region (the two arms) that interract with and attach to the antigen.

When the antibody sticks to the antigen it neutralises it so that the foreign organism can no longer enter a human cell or cause harm (see Figure 15).

Once an infectious agent is neutralised it dies.

Figure 14: Diagram of a Y-shaped antibody

The variable region - the antigen binding site (in yellow) and the constant region (in light blue)

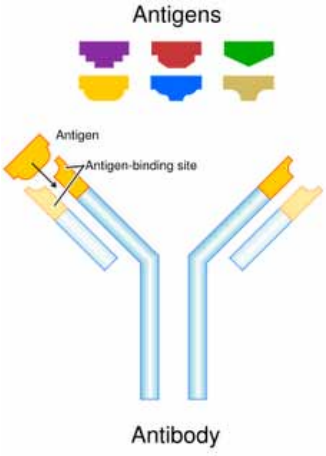
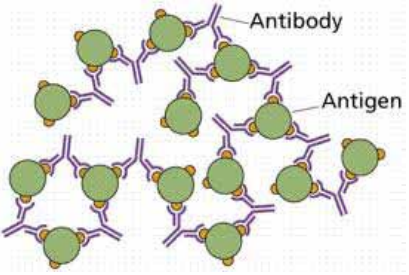


Figure 15: How antibodies and antigens interact

Antibodies neutralising an infectious particle.



How do antibodies and antigens interact?

One way to explain how an antigen and antibody interact is to compare them to a lock and key. The antigen acts like a lock and the antibody like a key. Each key is different for each lock.

As we grow, especially in childhood, we develop a library of millions of different antibodies. This makes up our acquired immune system.

This is a huge reference bank of immune cells that are generally resting or sleeping until they are needed.

Most HIV tests are based around this interaction. On the surface of HIV there are lots of proteins which act as antigens. One of the most common in early infection is called protein 24 (p24).

Antigens for HIV are detectable in most people around 16 days after infection.

Antibodies take longer to produce and are not usually detectable until 4-12 weeks after infection.

How does an ELISA test detect HIV antibodies?

ELISA tests are the standard test for finding out if someone has antibodies to a particular antigen. ELISA stands for enzyme-linked immunosorbent assay.

It is performed in 4 steps as shown in Figure 16.

Rapid tests

Rapid tests are a simplified version of antibody ELISA tests. They look for HIV antibodies in the blood. The antigens for HIV are fixed on one particular strip along the rapid test stick. Towards the end of the testing stick are control antigens to show that the test worked.

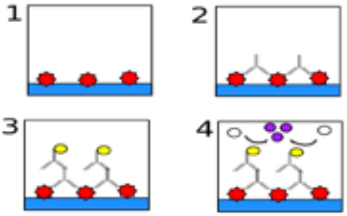
A sample is placed at the end of the testing stick. A chemical, called a buffer, to facilitate the testing process is added.

The chemical causes the antibodies in the blood to flow along the test stick. When they pass over the section with the antigens, if there are any antibodies for HIV present then they will stick to these antigens and change colour.

Once the test is complete, if there is one stripe it means it is a negative result. If there are two stripes then it means it's a positive result. If there are no stripes it means the test did not work properly.

This is illustrated in Figure 9 on page 29.

Figure 16: ELISA antibody detection test

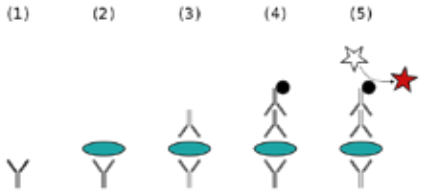


1. HIV p24 antigens are manufactured and attached to the bottom of a plastic testing dish.
2. The dish is then washed with the blood sample. If antibodies (upside-down Y-shapes) for the p24 antigen are present then they attach to the antigens in the dish. This gives an HIV positive result. If antibodies are present the steps 3 and 4 follow. If there are no antibodies, only antigen remains and this is a negative result.
3. Once the antibodies are attached to the p24 antigen, they need to be made visible. To do this the dish is washed with a second 'marker' antibody. The second antibody is specific to all human antibodies. When it comes into contact with a human antibody it will attach itself. This second antibody has an invisible 'marker' attached to the end of it (represented by the black circle)
4. Finally the dish is washed with a dye. Where the second 'marker' antibody is present, the marker (represented by the light circle) will cause the liquid in the dish to change colour (represented by the dark circles). Where there is no 'marker' antibody the liquid will remain clear. This means that any dish that is coloured is a positive result and any dish containing clear liquid is a negative result.

How does an ELISA test detect p24 antigen?

An ELISA to detect antigens is called a sandwich ELISA and works in a similar way (see Figure 17).

Figure 17: ELISA antigen detection test



1. Antibodies for p24 (represented by the Y-shaped picture below the number (1)) are secured to the bottom of a Petri dish.
2. The blood sample is added to the dish and if the sample is HIV positive then the p24 antigen on the surface of HIV (represented by the disc) will attach to the antibodies. If the blood sample is negative then there will be no antigens and the dish remains the same as in number (1)
3. An antibody specific to the p24 antigen is then added to the dish and where the p24 antigen is present it attaches. If there is no p24 antigen present then it washes away
4. Once the antibodies are attached to the p24 antigen, they need to be made visible. To do the dish is washed with a second 'marker' antibody. The second antibody is specific to all human antibodies. When it comes into contact with a human antibody it will attach itself. This second antibody has an invisible 'marker' attached to the end of it (the small dark circle)
5. Finally the dish is washed with a dye. Where the second 'marker' antibody is present, the marker (represented by the

black circle) will cause the liquid in the dish to change colour (represented by the stars). Where there is no 'marker' antibody the liquid will remain clear. This means that any dish that is coloured is a positive result and any dish containing clear liquid is a negative result.

Third generation tests are an ELISA that looks for an antibody alone. Fourth generation tests use both of the above methods to look for both antibodies and antigens.

What does the number after the HIV ELISA test result mean?

Some people are given test results which say something like 'non-reactive (OD: 0.219)'. This number at the end is called the Optical Density (OD) value. This is the measure of how much colour there is in the dish at the end of the ELISA.

As the colour in the dish is an indicator of whether the result is negative or positive these numbers give the result more precisely than just a simple 'positive' or 'negative' answer.

The cut-off values for different tests vary. In general, any numbers below 1.0 mean it's a negative result. Any numbers above 1.0 mean it's a positive result.

The numerical results of HIV tests are not related so if someone has 2 tests and the numbers look like they are increasing it does not mean they are slowly becoming positive. It is just two separate figures.

If someone has an 'inconclusive' test result it is possible that the OD is very close to 1.0 and a confirmatory test will have to be done.

How does a western blot test work?

The western blot, is similar to the ELISA in that it also detects antibodies for HIV. However, it works slightly differently to an ELISA. A western blot works by detecting antibodies to lots of specific proteins (antigens) at the same time.

To do the test, HIV is split into its various component proteins which are all different lengths and thus different weights (measured in kD – kilo Daltons). A blood sample is then mixed with the proteins and any antibodies for HIV in the blood sample attach to the proteins in a similar way to that in the ELISA outlined above. The antibodies present are then tagged using 'marker' antibodies (see ELISA section).

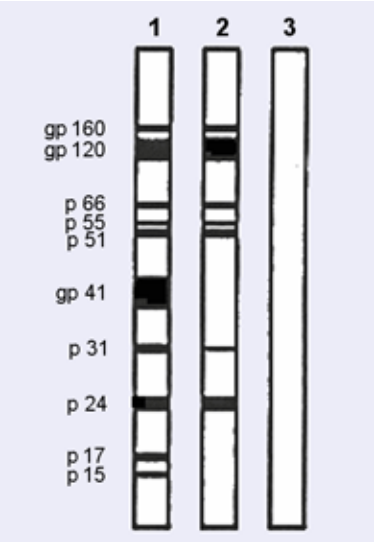
Each sample is then added to a dip made in a special gel. The gel is then plugged into an electric current and the proteins start to move down the gel.

The heavier proteins stop quicker than the lighter ones. The more there are of each protein, the thicker and darker the stripes. The gel is then developed in a similar way to a non-digital photograph to show which proteins are present.

If there are stripes where the HIV proteins should be then the result is positive. If there are no stripes then the result is negative.

The difference between the western blot and the ELISA is that the western blot can identify antibodies for lots of different HIV proteins or antigens at the same time whereas the ELISA will only look for one at a time. As shown in Figure 18 and 19.

Figure 18: A drawing of results of a western blot test



In this case Test 1 is a control as it shows all the proteins tested for are present.

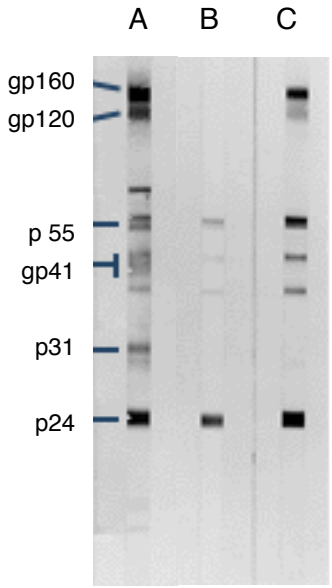
Controls are crucial to make sure that the test is working correctly.

Test 2 is a positive result as it shows there are antibodies for 7 of the HIV proteins.

Test 3 is a negative result.

Figure 19: Western blot test result

In reality a western blot does not look as neat as Figure 18 but looks more like the stripes below.



A - control strip showing antibody responses to key HIV proteins

B - Indeterminate response showing antibodies to p24

C - Positive western blot showing responses to at least three key proteins

PCR tests: DNA and RNA (viral load)

The polymerase chain reaction (PCR) test looks for genetic material from the HIV virus itself.

This genetic material can be RNA (single strand) or DNA (double strand).

RNA and DNA are long chains of chemicals. Different sections of this genetic material are like recipe books for making new virus.

A sample is amplified many times so that there is enough RNA or DNA to be measured.

DNA tests are used for testing babies born to HIV positive mothers and results are positive or negative.

RNA is used for viral load tests for most adult testing, including monitoring HIV positive people before and after treatment.

The PCR test produces a quantifiable result, which means that, as well as a negative/positive result, the amount of virus present in the sample can also be detected.

This result is given as copies per millilitre (copies/mL). The sensitivity of the test is usually 50 copies/mL, below which a result is referred to as undetectable.

How is the accuracy of HIV tests measured?

Accuracy of medical tests are often described in terms of:

Sensitivity - the percentage of the results that will be positive when HIV is present

Specificity - the percentage of the results that will be negative when HIV is not present.

The ideal test would have 100% sensitivity and 100% specificity, but few tests are ever this accurate.

Every diagnostic test has its limitations. On very rare occasions the results can be inconclusive or incorrect.

These are either *false positive* - the test result indicates that HIV is present when it is not or *false negative* - the test result indicates that HIV is absent in an infected person.

A second confirmatory test would eliminate this possibility. False negative and false positive results are discussed earlier in this booklet.

Stages of seroconversion

Stages of seroconversion and primary HIV infection by looking at the time that different HIV tests gave a positive reaction are shown in Figure 20.

Every one has their own immune system and response to infections. This table shows how difficult it is to say exactly when each test is accurate as there is such individual variation.

To help visualise the timeframe for the number of days after infection that each laboratory stage can pick up primary infection see Figure 20.

Figure 20: Stages of seroconversion when different laboratory tests can detect HIV

Stage	Test results +/- (positive/negative)	Timeline duration in days (95% CI *)	
		Individual time	Cumulative time
I	PCR +	5.0 (3.1, 8.1)	5.0 (3.1, 8.1)
II	PCR+ and p24+	5.3 (3.7, 7.7)	10.3 (7.1, 13.5)
III	PCR+, p24+, Ab+ (ELISA)	3.2 (2.1, 4.8)	13.5 (10.0, 17.0)
IV	PCR+, p24+/-, Ab+, WB indeterminate **	5.6 (3.8, 8.1)	19.1 (15.3, 22.9)
V	PCR+, p24+/-, Ab+, WB determined 2 out of 3 of p24, p41, p120; p31-negative	69.5 (39.7, 121.7)	88.6 (47.4, 129.8)
VI	PCR+, p24+/-, Ab+, WB full including p31+	Open-ended	Open-ended

(adapted from Fiebig et al. AIDS 2003).

PCR = viral load; Ab=antibody; Ag=antigen; WB=Western blot.

* CI (Confidence Interval) – this means there is a 95% chance each stage will fall into this timeframe e.g. that stage I will happen between 3.1 and 8.1 days

** Indeterminate means there would be an inconclusive result e.g. there is a strip but it is still too light coloured to be a definite positive (see Western blot section above)

Figure 20: HIV infection, immune responses and window period for different tests