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The HIV Seronegative Window Period: Diagnostic Challenges and Solutions

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1. Introduction

1.1 The phenomenon of the HIV seronegative window period

An exposure of an individual for the first time, to an infectious agent or any other foreign antigen, usually leads to recognition of the foreign antigen by the cells of the immune system (priming) followed by the generation of antibodies that specifically react with the foreign antigen. The process of generating detectable levels of antibodies against a new antigen/pathogen usually requires ~7 days following the initial exposure and infection.

In HIV infection there is a longer time lapse between infection and seroconversion. This time is termed the seronegative window period (WP). If an HIV antibody test is performed during the WP the result will be negative. However, the infected person is infectious and could potentially transmit HIV to others during this time. People taking an HIV test are advised, if the result is negative for HIV specific antibodies, to return for follow-up testing in 2-3 months.

The initial indications to the possible existence of a WP came from case reports where HIV seronegative individuals transmitted infection to others, by both the blood (blood transfusion, shared needles, medical practice), and the sexual routes (both homosexual and heterosexual). Pooling all those reported cases, coupled with mathematical modelling, has led the CDC to estimate the WP to be ~3 month (the time it takes 95% of the population to seroconvert following an HIV infection). This phenomenon, while considered as part of the "norm" in HIV infections, is actually quite surprising, as HIV is a very strong immunogen, and eventually it elicits high levels of a broad spectrum of antibodies against both envelope and core antigen. Clearly this WP is a major obstacle in the path of early and complete detection of HIV infection, and it is this challenge and the different solutions to it that are the focus of this chapter. However, when trying to overcome this hurdle, it behoves us to try and understand the immunological enigma of the WP, and its causes; as understanding the roots of the problem is usually a major part of the solution.

1.2 Using the monkey model of AIDS to study the HIV seronegative window period
1.2.1 The monkey model of HIV infection

The monkey model of AIDS, i.e. the Simian immunodeficiency virus (SIV) in monkeys, has been used for research of the different aspects of the HIV infection and its pathological sequllea, including the interactions between the immune system and the virus at different

stages of the infection. There are two main groups of monkeys in this model – those who are natural hosts of SIV, and those who can be experimentally infected by it. The African monkeys (Sooty Mangabeys) are among the natural hosts for SIV (they develop high viral titres but do not show any signs of SIV related disease throughout their life).

When virus from Sooty Mangabeys (or other African monkeys) is transmitted to naïve Asian monkeys (e.g. Macaques) it leads to an SIV infection with an infection course and pathological sequella similar to that of HIV in humans. Thus this serves as a good animal model for studying HIV infection and AIDS. The time to seroconversion in the Asian monkeys is similar to that observed and estimated in humans. Of course, unlike in natural infection, the length of the WP can be accurately measured in experimental infections. Studying the immune response against SIV in the Asian rhesus macaques, who upon experimental infection with SIV develop disease and clinical symptoms remarkably similar to human HIV-1 infection, could shed some light on the WP as part of the common course of the HIV infection and maybe on the longer seronegative state too.

1.2.2 The seronegative WP in SIV infection

Most of the young Sooty Mangabeys remain seronegative for 2-3 years, eventually seroconverting. As the seroconversion age range coincides with sexual maturity, the dogma has been that these monkeys become infected via the sexual route. However, there have been several reports indicating that they might be infected throughout the seronegative early years of their lives (Jehuda-Cohen et al. 1991; Villinger et al. 1991) and potentially even from birth (Jehuda-Cohen et al. 1991; McClure et al. 1991). If infection occurs in utero or at birth, then the seroconversion upon sexual maturity could be attributed to "new" SIV stimulus or re-infection, which would change the immunological state and lead to stimulation and generation of a measurable antibody response. One possible model for explaining the whole phenomenon could be some form of peripheral tolerance to SIV induced by the in-utero exposure, which would be "broken" when similar but not the same antigen enters the system via the sexual route. Interestingly, in a colony of several dozens of seronegative magabeys, which were kept, as adults, in a separate, seronegative colony for over 8 years, an alpha male sero-converted, for reasons yet to be determined. This could suggest that these natural hosts of SIV may have been infected for a long time and thus had a very long WP (Villinger F. personal communications).

In experimentally infected Asian monkeys, a seronegative window period of more than several weeks is more rare. However, there has been cases of low dose infections where the monkeys remained in a seronegative state for months and years (unpublished data). We have also reported a prolonged seronegative state in macaque babies following infection in-utero or soon after birth (Jehuda-Cohen et al. 1991).

1.2.3 Exposed seronegative (ESN) individuals from high risk groups

Some individuals residing in very high risk populations have been reported to remain seronegative for many years in spite of repeated exposures to the virus. While this WP has been known to be highly variable, the precise mechanisms that give rise to this long seronegative WP have yet to be defined. It is not clear whether the 'exposed seronegative' [NIH workshop 2010 on ESN] is a unique phenomenon, separate from the common seronegative WP, or it could be viewed as the far extreme end of a possible spectrum of the length of that WP. In any case, it has been reasoned that delineation of the mechanisms

underlying this phenomenon could provide important clues for effective vaccine formulations.

While studies of these cohorts of ESN people are of interest, they do not shed any light on the early immunological events following the HIV infection, as we see in the ESN only the end result (G. Shearer, personal communications). The SIV infected nonhuman primates, might be able to provided a valuable model to study this issue too.

There have now been several reports that a small but significant number of these rhesus macaques when experimentally infected with repeated low doses of SIV intra-rectally or intra-vaginally become highly resistant to infection with a dose of challenge virus that otherwise leads to 100% infection of this species. Of importance is the finding that these highly resistant rhesus macaques neither sero-converted nor developed any detectable SIV specific cellular immunity (Ansari A. personal communications).

1.2.4 Immunological findings of the early stages of infection

The mechanisms underlying the seronegative WP are complex and studying them is encumbered by the fact that we do not have a way to identify those who are in the eclipse period. Thus we are left with three main routs to study this very critical time in the HIV infection, i.e. its onset: 1. Set up large scale studies where fresh blood samples, from HR cohorts, are collected longitudinally for months and years, so that when there is a seroconversion, it is possible to have, retrospectively, good samples (e.g. cryo-preserved cells) to study those early days in the HIV-human-natural setup. 2. To run the immunological and virological studies on samples from the acute phase of the infection and the early days post seroconversion. 3. To use the monkey model of AIDS, in which the time of SIV experimental infection is known, and the virus, its dose, the route of infection, the rate and type of repeat exposures/infections can all be controlled.

The first option is extremely expensive and labour intensive, and rarely attempted, as the vast majority of the samples processed for PBMC etc, will be of those who will remain seronegative through out the study. There has been much work published using the 2nd route (Myron S. Cohen 2007; Margoli 2009), albeit they offer us a view into the post active viremia stage, leaving us in the dark as to the earliest days post infection. Such studies (Tomaras and Haynes 2009) have revealed that there is early destruction of B cell generative microenvironment, and that this might be one of the causes for a delay in protective anti HIV antibody responses (Richman et al. 2003; Davis et al. 2009; Stacey et al. 2009) and other parameters of antibody response (Tomaras et al. 2008). Polyclonal cell activation in early HIV infection and loss of gut germinal centres have been observed (Levesque et al. 2009). These findings of B cell depletion have been confirmed in acute human HIV infected individuals (Marovich M. personal communications). A rapid cytokine storm in acute HIV infection might also contribute to the lack of an appropriate maturing antibody response (Stacey et al. 2009).

Using the SIV model, it has been reported during acute infection, that there is not only a major depletion of CD4+ T cells but also a major depletion of B cells (Titanji et al. 2010). In a study comparing the early immune response to two different SIV strains, with different pathological outcomes, it was shown that: early systemic immune activation, T cell proliferation, and a more prominent and broader array of cytokine/chemokine responses facilitate SIV replication, and may play a key role in persistence of infection, and the progression to AIDS (Xu et al. 2011). This immune activation and SIV proliferation also leads to a fast depletion of T cells.

2. Potential causes and effectors of the length of the WP

Before we look into the potential effectors of its length, we should remember that, in fact, any WP in HIV infection is an enigma as HIV has very strong immunogenic structures, which eventually elicit a strong immune response against it. The CDC states that the length of the WP for HIV varies from country to country and from population to population, as it is affected by geographical, social, genetic and other factors, all of which affect the immune system of the host.

One such example would be the WP in pregnant women. Pregnancy has been purported to induce an altered immune state and some data suggests that certain infections may have worse presentations and outcomes during pregnancy (Landers et al. 1997). Following experimental HIV vaccination in Brazil, anti-HIV-1 immune response was the strongest in intravenous drug users (IDU) group and the weakest in pregnant women. A comparative analysis between pregnant women cohorts from different regions of Brazil indicated an even lower response for the southern population (Bongertz et al. 1998). So, unrecognized HIV infection just prior or during pregnancy may result in higher rates of prenatal transmission (Patterson et al. 2007). In addition, presence of parasitic worms is generally associated with immune suppression. A study on the impact of helminthes on the response to immunization in pregnant women and children in Uganda has shown that infection with helminthes has suppressive effects on the immune response (Elliott et al. 2007). General immune suppression in MSM would be another example, and in that population a very long WP has been reported (Imagawa et al. 1989; Gupta et al. 1992). Viral genotypes and clades (Andersson et al. 2005), social and environmental factors, and genetics, could also play a role in determining the HIV WP.

Studies in the SIV (monkey) model of AIDS were part of the research that enabled an understanding of some of the underlying viral and immunological mechanisms that lead to the seronegative yet infected stage of the infection. It has been discovered that the immune system "sees" the virus at the onset of the infection, gets primed by it, but due to specific immune suppression it does not lead to seroconversion (Powell et al. 1991; Jehuda-Cohen et al. 1994). Only several weeks or months later, when there are high levels of virus in the blood, antibodies are finally produced by the immune system at detectable levels in the blood. In a study among individuals who were at high risk for HIV, a similar suppression of antibody production was found leading to a seronegative state in spite of an HIV infection (Jehuda-Cohen et al. 1990).

Upon infection, the HIV seems to home to the lymphoid tissues, of which the leading one is the gut mucosal tissue. At that time there would be no, or almost no, virus detected in the blood. A link has been proposed between the time of active viremia in the blood reaching certain levels, and the time of seroconversion (Busch et al. 1995). The time between the infection and the active viremia reaching detectable levels in the blood is called the eclipse period. There has been reports of low, intermittent levels of detectable levels of virus in the blood (Fiebig et al. 2003) for weeks, until eventually the infection changes to an active viremia leading, usually, to constant measurable levels of virus in the blood, and to seroconversion.

3. Ways to shorten the window period and the time of "no-detection"

3.1 Better detection of antibodies

The WP has been a major concern in both the blood banks (and other tissue transplants) and in the diagnostic arena. While the detection of HIV specific antibodies in the plasma has

remained the gold standard for diagnosis, there has been much pressure to develop methods which could shorten the period of no-detection. The initial steps taken where to enable the detection of IgM antibodies in addition to the IgG antibodies, thus bringing to the market the 3rd generation assays for HIV specific antibodies. (The 1st and 2nd generation assays detected only HIV specific IgG). This, together with increased sensitivity has shortened the WP, yet the confirmation by Western blot kept the confirmed diagnosis delayed as before (Owen et al. 2008).

3.2 Detection of virus in the blood

The second approach was to address the issue of no-detection via the detection of the active viremia in the blood which precedes the seroconversion. Thus, methods which detect the presence of the virus in the blood (p24 antigen, viral RNA, or pro-viral DNA) have enabled the detection of the infection 7-12 days, respectively, prior to seroconversion. This improved detection has enabled the detection of RNA, prior to seroconversion in 0.3% of 14,005 frequently tested MSM in Seattle STD clinic (represents 20% of all HIV infections detected), (Stekler et al. 2009); and in 0.08% of 21,222 STD clinic patients in New York City (represents 9% of all HIV infections detected), (Shepard et al, MMWR in press).

Linear regression analysis of the detectable part of the active viremia stage led to an estimation that the "beginning" of the active viremia in the blood was 10 days prior to the virus reaching detectable levels in the blood. Thus the total length of the active viremia prior to seroconversion was estimated at 22 days (Busch et al. 2005). The detectable part of the active viremia prior to seroconversion i.e. the virus positive yet antibody negative plasma is called the "acute stage" of the HIV infection. The part of the active viremia which is not detectable, and the period prior to the active viremia, when the virus reside in the mucosal membranes of the gut (Brenchley and Douek 2008; Mestecky et al. 2009), in lymph nodes (Pantaleo et al. 1994; Schacker 2008), macrophages in the lung and other tissues (Orenstein 2001), is termed the eclipse period.
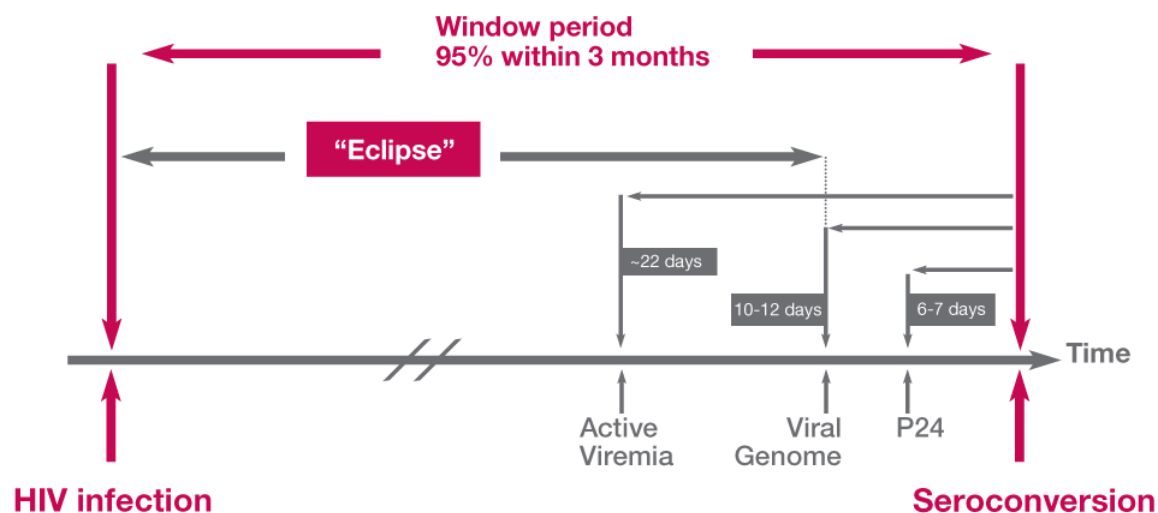

Fig. 1. Time line from HIV infection to seroconversion.

3.3 Dealing with the root of the problem.

Since the mechanisms underlying the seronegative WP include specific immune suppression, other factors affecting the immune state of the person could affect the length

of the WP. Such conditions include pregnancy, parasitic infections and other co-infections, MSM relationships, hemodialysis, and others. The variability in the length of the WP also means a variable length for the eclipse period, i.e. the period in which the HIV infection is totally missed by all the currently available assays in plasma. Thus the optimal way to solve the WP problem would be to be able to overcome the immune suppression and lead to antibody production, in-vitro, soon after the initial HIV infection and immune priming in-vivo.
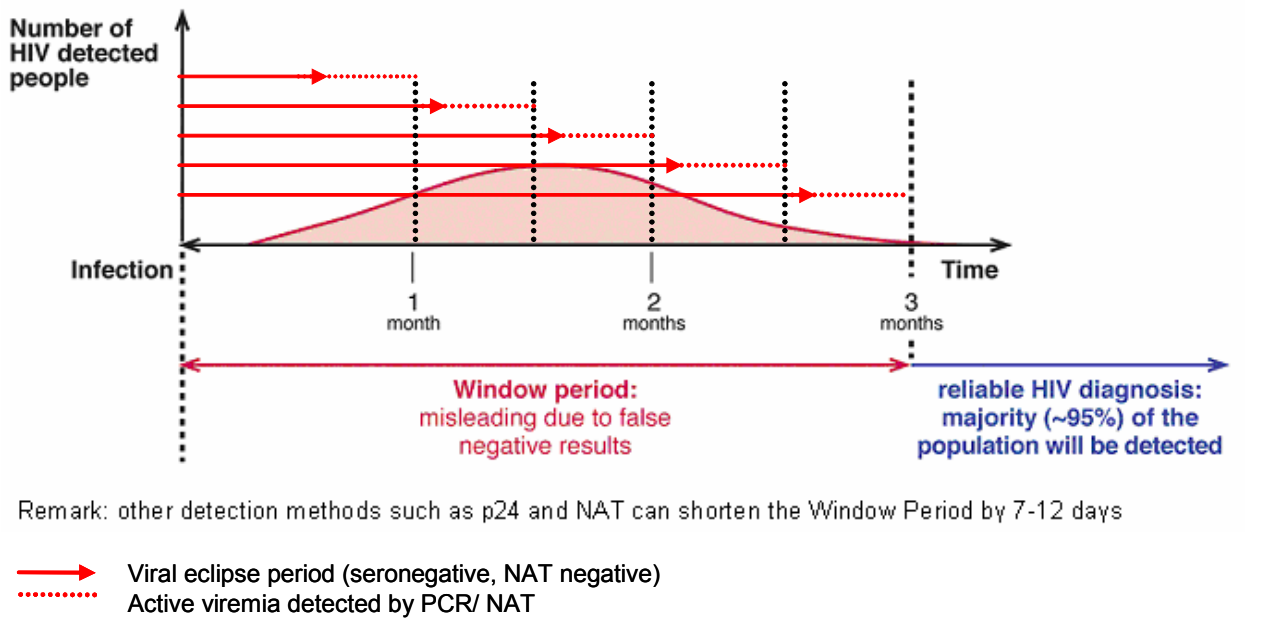

Fig. 2. An illustration of the statistical distribution of the length of the seronegative WP in a population, and the relative length of the viral eclipse period.

4. The Stimmunology concept and the SMARTube HIV

Since the immune system "sees" the virus, and its lymphocytes, both T and B cell, get primed by it within minutes of infection, tapping into these early events could enable the detection of HIV infection within days. A method was developed to enable the process which has been initiated in-vivo (specific cell priming) to be completed in-vitro, by leading to cell proliferation and differentiation and to HIV specific antibody production, in the case of an HIV infection. The stimulation in-vitro is designed to overcome the immune suppression in-vivo and to provide the lymphocytes in the blood sample a strong stimuli to produce antibodies in culture.

This unique and innovative technology (called Stimmunology) has been shown to enable the detection of HIV infection within a week after exposure, several weeks or months prior to seroconversion. Detecting infection via the specific antibodies in the blood is routinely used in any clinical laboratory and seropositivity to HIV is considered the gold standard in diagnostics. The sample used in these assays can be serum or plasma with or without pre-stimulation.

The embodiment of Stimmunology into a simple to use product is the SMARTube™, which requires one ml. fresh whole blood. Blood, when introduced to the SMARTube, is

stimulated so as to enhance the synthesis of HIV specific antibody, and the differentiation of HIV primed B cells to antibody producing cells. The resulting plasma, enriched with antibodies via the tissue culture step, is called SMARTplasma. SMARTube accelerates antibody production bringing the antibody levels, in the SMARTplasma, across the regular ELISA testing's detection threshold, leading to earlier, better and more complete detection and diagnosis of the HIV infection.
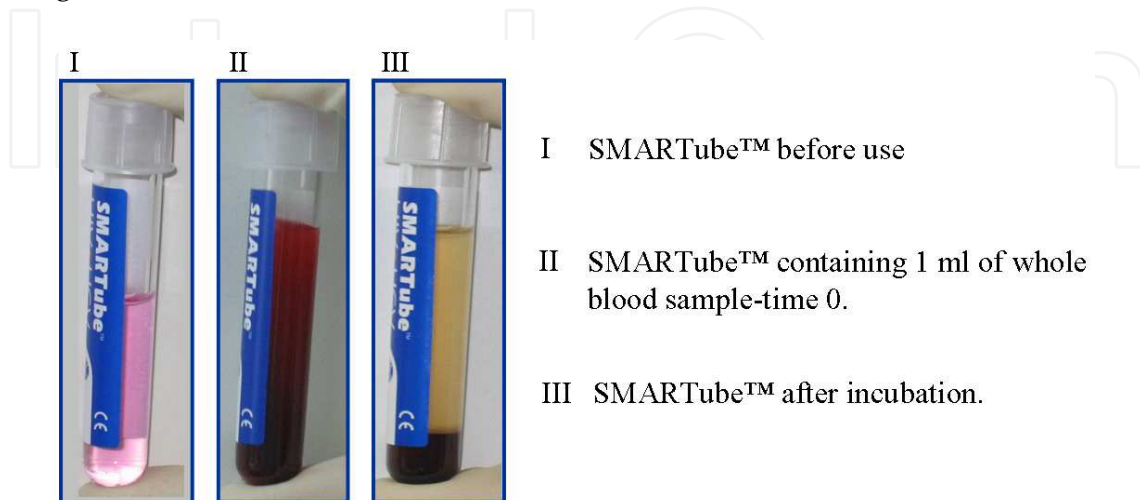

I   SMARTube™ before use

II   SMARTube™ containing 1 ml of whole blood sample-time 0.

III   SMARTube™ after incubation.

Fig. 3. Getting SMARTplasma from fresh whole blood by incubation in the SMARTube for 3-5 days in a 37°C, 5%CO_2 incubator.

5. SMARTube enables detection of antibodies prior to seroconversion

Comparative laboratory studies were conducted in different populations and different geographical locations, testing plasma and SMARTplasma in parallel, from the same blood sample, using locally approved diagnostic kits. The confirmation of an initial antibody positive test results (of plasma and/or SMARTplasma) was done following the local guidelines and algorithms; thus differentiating between true antibody positive samples and false positive ones.

In China, among 653 IDU 149 were confirmed seropositive (antibody positive in plasma) and 2 (1.3% additional HIV positives) additional individuals were confirmed antibody positive in SMARTplasma, enabling detection of the HIV infection prior to seroconversion [Dr. Wang Y. personal communications]. In a parallel study, conducted using 2000 low risk individuals from the blood donors of Beijing blood bank, no additional positives were found using the SMARTube, showing no adverse effect on specificity. It was further documented that when testing SMARTplasma there was a marked reduction in the rate of false positive readings, in both diagnostic kits used [Qui W. personal communications] When blood donors from a high prevalence, high incidence population (Kenya) were tested for HIV infection, there was a high rate (4%) of missed HIV infections, detectable by the antibody diagnostic kits only after incubation of the blood samples in the SMARTube (Mumo et al. 2009). Viral testing was done on the seronegative WP samples detected using SMARTube, and virus was detected in ~50% of the SMARTplasma positive seronegative. This further confirms the fact that the SMARTube is not dependent on detectable levels of virus in the blood in order to enable the detection of very early infection, i.e. with it HIV infection can be detected even in the viral eclipse period.

An immigrant group, coming from a high risk country into Israel, where the risk was through sexual transmission, was also tested (Novikov and Jehuda-Cohen 2009). In both waves of immigration additional HIV antibody positive infected individuals were detected. In the first wave of 285 tested, 8 of the 15 infections were in the WP, while in the second wave of the 537 tested, 2 of the 28 infections were in the WP. The difference between the two populations was that the first population had been exposed to high prevalence and high risk of HIV only for one year, which explains the lower prevalence and the higher number of the infections being recent ones, with many still in the seronegative WP. The second population was exposed to HIV for several years, leading to a higher prevalence but a lower number of new infections which were missed by current serology.

Country	Population studied	Total tested	ST negative		ST positive*	
			Serology negative	Serology positive*	Serology negative	Serology positive*
High Risk						
Israel	*Immigrants from high risk areas, high risk population first wave*	285	270	0	8	7
	Immigrants from high risk areas, high risk population 2nd wave	537	519	0	2	26
Mexico	*Very high risk population, multiple, current exposures*	200	175	0	5	20
Kenya	*Screening of high risk population*	555	513	0	14	28
	Adult blood donors	513	447	0	21	45
	Youth blood donors	332	310	0	10	12
	Seronegative Pregnant women	20	8	0	5	7
China	*High risk population (IVDU), Sichuan District*	653	502	0	2	149
South Africa	*Blood donors from high risk population*	90	85	0	2	3
Russian Federation	*Discordant couples, high risk population, Blood donors,*	24	18	0	1	5
Hungary	*Routine Laboratory tests*	41	40	0	0	1
	National AIDS center	203	55	0	1	147
Romania	*Routine Private Laboratory tests*	200	200	0	0	0
Low Risk						
China	*Low risk population, Beijing Blood Bank*	2000	2000	0	0	0
Russian Federation	*Low risk population, Blood donors*	25	25	0	0	0
Israel	*Low risk population, Blood donors*	1500	1500	0	0	0
* Positive = confirmed by repeat testing.						

Table 1. Increase in HIV antibody levels, using the SMARTube, led to earlier detection of HIV infection (i.e. detection of seronegative, yet infected, individuals)

In Russia, 25 discordant couples were tested using the SMARTube. Five were seropositive on both plasma and SMARTplasma, however there was an infected person who tested positive only when using SMARTplasma (Olshansky 2008). Viral load was 900,000, i.e. that

person was not only HIV infected and still in the WP, but also very infectious, and missed by current serology. When the SMARTube was incorporated into routine laboratory use, within the first 300 samples tested, the confirmed diagnosis of one patient was achieved, using SMARTube, 4-6 weeks prior to complete seroconversion in plasma (Bicbulatova et al. 2010).

In South Africa, in a high prevalence and incidence area, a cross sectional comparative study showed full concordance between the confirmed antibody positive results in plasma and in SMARTplasma. In a prospective study, several hundred individuals were followed, monthly for up to 9 months, to measure the rate of new infections by seroconversions. In several individuals, antibodies were detected in SMARTplasma 1-4 months prior to plasma seroconversion [Sexton C. personal communications].

It is important to note, that while the incubation of the blood in the SMARTube increases the levels of the HIV specific antibodies in infected individuals, it does not adversely affect the diagnostic specificity. On the contrary, the SMARTube has been found to decrease the false positive rate on the routinely used diagnostic kits, thus increasing the specificity of the kit in the tested population (Mumo et al. 2009; Novikov and Jehuda-Cohen 2009, and unpublished data). There are several mechanisms which contribute to this phenomena, one of them being that while increasing the specific signal (HIV antibodies) the plasma itself is diluted 1:5 (1ml of blood, i.e. ~0.5ml plasma, put into 2ml of SMART solution), thus decreasing "noise" and leading to a decrease of as high as 100% in the false positive rate.

In addition, the use of the SMARTube enables the laboratory to get, and provide, a more confirmed negative result. Currently, using plasma, those who were seronegative, yet in the WP, (i.e. actually infected) are falsely recorded as negative. One cannot differentiate between those who are truly HIV negative and those who are HIV infected yet still in the WP – they all give the same 'negative reading' on the assays used. When using the SMARTube, the WP samples test positive, thus making the antibody negative results confirmed negative (see Fig.4).
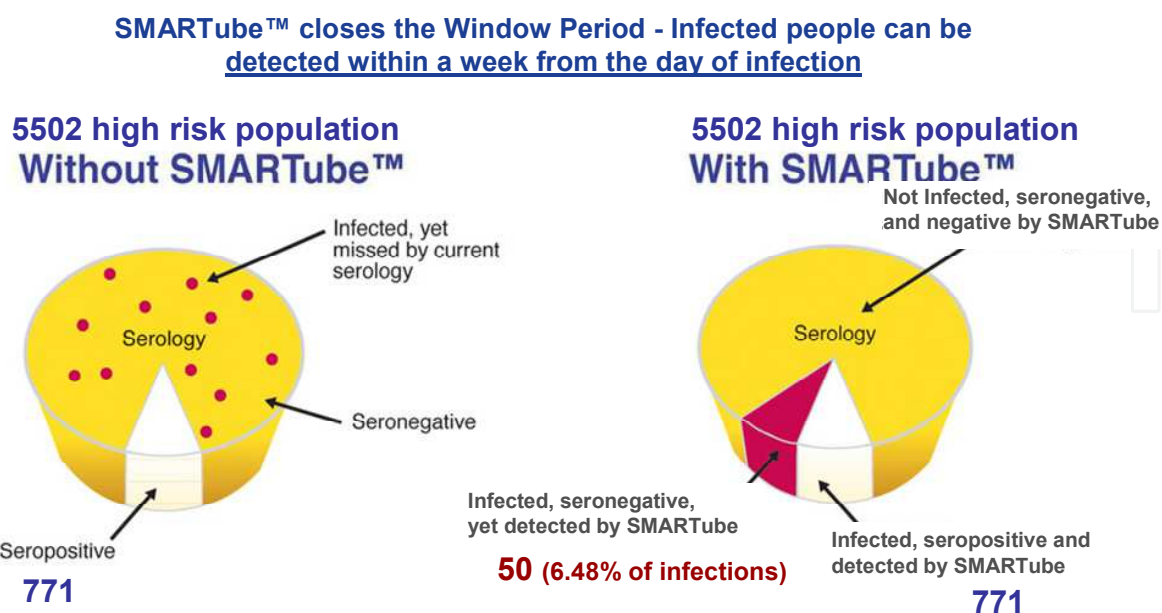

Fig. 4. Consolidated results from laboratory clinical data in different studies using SMARTplasma (following incubation in the SMARTube) in parallel to regular plasma

6. The importance of early detection of HIV infection

6.1 Infection at the seronegative WP is infectious

Over the course of the HIV epidemic, there have been reports in literature of individual who tested negative for antibodies yet were infected and infectious, leading to the infection of others. Long term seronegative yet infected state has been reported, especially once more sensitive methods for detecting the virus were developed (Imagawa et al. 1989; Ensoli et al. 1990; Gupta et al. 1992).

Incomplete detection of potentially infectious blood units (Ling et al. 2000), and other donated organs (e.g. sperm, kidney, bone marrow) can transmit HIV infection to the recipients. Thus the need to shorten the window period, by as much as possible, has been an important goal of the health systems around the world. The importance of blood safety and of transplanting organs which are free of HIV (and HCV and others) infection have been major engines in the introduction of viral (RNA) testing into blood screening and into organ donors' testing. The cost of NAT testing of the single, individual, organ donor is acceptable. The cost at the blood bank level had to be dramatically reduced, and this was done by testing the blood in pools of 16, 24, 40 and more blood units/pooled test. The gain by the pooled testing, and the loss of sensitivity due to the pooling has been the topic of many studies and reviews, and is not in the scope of this chapter.

6.2 Infectious blood and blood products from donors at the seronegative WP and the viral eclipse

The transmission of HIV via blood transfusions was markedly reduced by the introduction of NAT testing to the blood banks, yet there have been several cases of HIV transmitted through a blood donation, in the USA, in spite of the testing for both antibodies and viral RNA (limit of detection 150 copies/ml). Such cases are termed HIV breakthrough cases (Delwart et al. 2004). In some cases one WP blood donation infected two recipients (Taylor et al. 2002). In Germany, in 2007, a 67 year old man got HIV from a screened blood donation
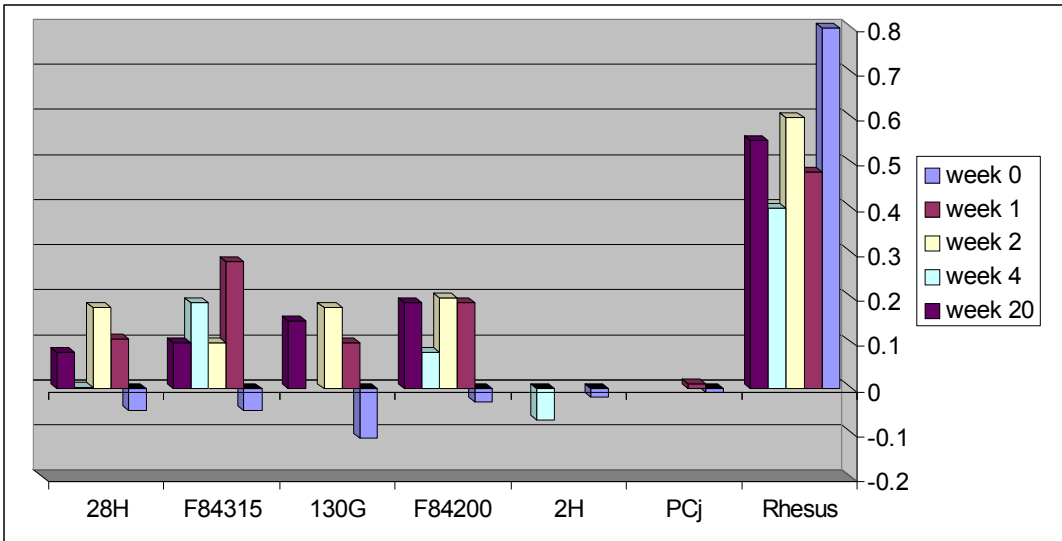

Fig. 5. SIV-reactive antibodies following Stimmunology – detection in the first week of infection (28H, F84315, 130G, and F84200 were transfused. 2H and Pcj were negative controls. Rhesus is a seropositive monkey).

(Schmidt et al. 2009). In Singapore, it has been reported that a blood transfusion was the source of an HIV infection. The authors state that the blood donation and transfusion of blood components occurred in Singapore, where blood donation testing for HIV is similar to US protocols, including anti–HIV-1 and anti–HIV-2 EIAs and an HIV-1 p24 antigen EIA (Ling et al. 2000). In another report two infections were caused by a blood donation in 2002 (Phelps et al. 2004). The donor was an adolescent repeat donor who tested HIV antibody positive in May 2002, and the previous donation (3 months earlier) must have been during the seronegative WP and at the eclipse of the virus too.

A study in monkeys (SIV) to address the question of infectiousness of blood from seronegative monkeys (sooty mangabeys), yet positive for SIV antibodies after cell activation in-vitro showed 100% infectivity as 4/4 of the naïve macaques, transfused with the seronegative mangebeys' blood, seroconverted 1-3 moths following the transfusion (Jehuda-Cohen et al. 1991). At the same study, it was possible to compare the WP using plasma versus the detection of antibodies following the Stimmunology process. All 4 transfused monkeys (Fig. 5.) had detectable levels of SIV antibodies following the stimulation process at the first bleed, one week post-transfusion (3-11 weeks earlier).

6.3 Organs from donors at the seronegative WP and the viral eclipse – transmitted infection

The relevance of the following cases, reported in literature, and mentioned here as examples, is not in their number and prevalence but rather for the light they shed on the WP, which even during the viral eclipse could be infectious, and thus of a major diagnostic concern.

In a report of a liver transplant, which transmitted HCV (Ahn and Cohen 2008) the authors summarize cases of HIV transmission through solid organ transplantation between 1985-1987. This problem was much more pronounced then, when only antibody testing (1st and 2nd generation) was available. However, shortening of the seronegative WP by more sensitive ELISA, and by 3rd generation ELISA, and adding the viral RNA testing (for a 10-12 days shorter WP), did not solve the problem completely (CDC 1987; Simonds et al. 1992; Mitra 2004).

Two related cases of infectious donations are described from a cornea donor during the pre seroconversion window (Najioullah et al. 2004). HIV was transmitted via a kidney transplant from a cadaveric donor (Borchi et al. 2010). In 2007, it was reported that, four transplant recipients in Chicago have contracted HIV and hepatitis C virus from an organ donor (Grady 2007). The organ donor tested negative for both viruses, both in antibody and in viral genome testing and apparently the donor was still in the seronegative WP and the viral eclipse period and thus the infection was missed. Following this case estimation of the window period between infection and detection by ELISA assay and NAT testing for HIV, hepatitis B virus, and hepatitis C virus was published (Singer et al. 2008). The CDC reported a case of infection via a kidney transplant in NYC in 2009 (CDC 2011). The donor was in the seronegative WP. Another kidney transplant HIV infection has just been reported this year by the USA health administration.

6.4 Individuals at the early stages of infections are the most infectious

Statistical analysis of epidemiological data indicates that the majority of new infections are transmitted from the small % of individuals who are in the early stages of the infection and that the acute infections are the most infectious (Pilcher et al. 2001; Pilcher et al. 2004; Wawer

et al. 2005). An analysis of HIV-1 Transmission, by stage of infection, indicated that primary infection was estimated to be 26 times more infectious than asymptomatic infection. High infectiousness during primary infection was estimated to last for 3 months after seroconversion. Thus we have 4-5 months of very high infectiousness, with 25-40% of that time the infection is in the seronegative WP (Hollingsworth et al. 2008). In 2007, it was reported, based on a North American urban study, that primary/early infection, representing <10% of the samples, disproportionately accounted for approximately half (49%) of onward transmission events" (Brenner et al. 2007).

Recent primate data demonstrate marked enhanced infectiousness of viral variants isolated from acutely infected macaques compared with viruses isolated from animals in the chronic phase of disease. These data are supported by phylogenetic analyses of recently transmitted cases in humans, implying that individuals with Primary HIV infection (PHI) may contribute disproportionately to onward transmission at a population level (Hamlyn 2010).

Other studies have shown that those who are unaware of the sero-status are 3.5 times more infectious than those who are aware (Marks et al. 2006). The CDC has reported that the high proportion of MSM unaware of their HIV infection continues to be a serious public health concern, because these MSM account for the majority of estimated new HIV transmissions in the United States (CDC 2010). One can only stipulate, on the effect of false negative results given to individuals who suspected infection for reasons best known to them. A false negative result could remove the final deterrent from behaviors which could set them at risk of HIV transmission. False-negative, WP, people do not take precautions or listen to preventive guidelines.

6.5 Pregnant women in the WP, a missed opportunity to treat and save the babies

The problem of the seronegative WP is especially critical in pregnant women. On one hand, the infection, which could have been transmitted close to the time of pregnancy, could have a longer WP due to the general slight immune suppression induced to preserve the fetus. On the other hand, there is usually a one time chance to save the fetus from infection, reducing the risk of mother-to-child-transmission from 28-30% to 1-2%, by giving the mother a short course of ARV during pregnancy and child birth (Cooper et al. 2002; WHO 2006). That chance is during the initial (and sometimes only) visit to the doctor or antenatal clinic. However, the provision of ARV is contingent on the expecting mother testing antibody positive, thus excluding from the treatment those who are in the WP at the time of testing (Dao et al. 2007). Thus the efficacy of the PMTCT programs, which include HIV testing to all pregnant women and ARV to all those who are HIV seropositive, depends, among other factors, on overcoming the seronegative WP to get early and complete detection of the infected mothers (Workowski and Berman 2010).

In a study conducted among very high risk pregnant women in Kenya, antibodies were detected in SMARTplasma 2.5-5 months (mean time between last seronegative and first seropositive sample) prior to detection in plasma (Mumo et al. 2009).

The long WP in pregnant women could have, in some rare cases, another ramification – missed infections in the new-born. Babies born to seronegative mothers, even from a high risk population, are not suspected to be infected. However, children born to (seronegative yet infected) mothers in the WP could get infected. This phenomenon has been documented in monkeys (SIV) and in unique case reports in HIV (Jehuda-Cohen et al. 1991; Jehuda-Cohen et al. 1992).

In Botswana, there is a state wide PMTCT program. In 2007 nearly all pregnant women (>95%) had antenatal care and delivered in hospital, and ~80% of pregnant women were

tested for HIV (Creek et al. 2007), and by 2007 91% of seropositive pregnant women received ARV (NACA 2007; 2008). However, the rate of MTCT has not been reduced to the expected level of 1-2% and remains at ~4%. It is thought that some of the unresolved MTCT are partially due to missed infections due to the WP.

Patient ID	Sample date	1st Ab test Plasma	1st test SMART plasma	Repeat Ab test Plasma	Repeat Ab test SMART plasma	p24 Ag	SMART plasma WB	Seroconversion after SMART plasma +Pos result (mid-point)
ML1232	0	0.469	**2.457**		**2.325**	**1.914**	**pos**	
	3 Months	0.350						
	5 Months	**18.750**						**4 Months**
ML1326	0	0.450	**2.871**		**3.700**	0.677	ND	
	4 Months	**6.125**						**2 Months**
	10 Months	**10.181**						
ML1356	0	0.137	**1.579**		1.733	0.484	Ind	
	4 Months	0.044						
	6 Months	**6.187**		**8.150**				**5 Months**
TDD164	0	0.931	**5.650**	0.817	**4.667**	0.486	**pos**	
	5 Months	**1.375**		1.767				**2.5 Months**
	10 Months	**11.800**						
	12 Months	**11.031**						
ML1324	0	0.656	**1.607**		**2.300**	**2.263**	**pos**	
	2 Months	0.425						**Lost**
Ab test – Ab ELISA test; P24 Ag – ELISA test for p24 antigen; WB – Western Blot; Pos – positive; Ind. – indeterminate; Lost – lost to follow-up. Results are presented as a ratio Signal/Cut off								

Table 2. Follow up HIV antibody testing of Kenyan pregnant women from high risk population (ELISA O.D. readings are presented as a Signal/Cut off ratio)

Blood samples	Date of birth	Date of first SIV-seropositive sample	ELISA Ab titer	Supernatant fluid following Stimmunology (cutoff 0.120)	PCR results	RT activity by the co-culture assay
Mother 1 (FHe)	7/82	-	>1:10	0.28±0.03	+	18.711
Infant 1.1 (FYb)	8/86	11/88	1:8000	0.97±0.03	+	35.253
Infant 1.2 (FVj)	6/88	3/89	1:2000	0.68±0.05	+	22.498
Mother 2 (FOe)	1/83	-	>1:10	0.22±0.01	+	15.966
Infant 2.1 (FRi)	7/88	3/90	1:4000	0.89±0.04	+	24.595
Negative control	-	-	>1:10	0.01±0.01	-	452
Positive control	-	-	1:4000	0.76±0.05	+	31.211

Table 3. Results of sera and Stimmunology enhanced samples from two SIV-seronegative female monkeys with seropositive infants

6.6 Earlier detection could lead to earlier treatment

Test and Treat programs are being evaluated around the world. Treating as early as possible is both for the benefit of the infected individual (e.g. better prognosis, a potential for cure) and of society (as ARV reduces the viral load, and thus the risk of transmission to others). There are contradicting voices regarding implementation of Test & Treat to all seropositive individuals, regardless of how long they have been infected. However, with regard to treating early and acute infections, there is a consensus that it is beneficial both for the individual and the society. PHI refers to the initial phase (up to 6 months following acquisition) of infection characterized by a transient period of massive unchecked viral replication with consequent destruction of memory CD4 T-cells (Douek et al. 2002). There are some large scale studies on going to evaluate the benefits of ARV at the PHI state as part of the Test and Treat approach. Until these provide concrete proof for the benefit to both the individual and society (and in that order), the current world policy for treatment only after CD4 <350/ml would most probably not change (WHO 2010).

The goal of early intervention is to preserve immune function which is ordinarily lost, enhance rapid viral control, and limit the size of the HIV reservoir, with the aim of attenuating long-term outcome (May et al. 2007). Studies regarding the effect of HIV on the brain in the early stages of HIV infection have been recently published. One demonstrated that several markers of inflammation were higher in acutely infected people (Valcour V 2011). These changes were found as early as the second and third of four 'Feibig stages' prior to seroconversion (Fiebig et al. 2003). In another study HIV injury to the brain, affecting its structure, were seen as early as 2 months post infection. The early initiation of ART was supported by preliminary results showing a lesser effect in people on HIV treatment (Rangin et al. 2011).

Intuitively, the earlier that intervention can be initiated following HIV acquisition, the more enhanced will be the anticipated effect on outcome. Although much of the early immunological work has focused on acute infection (Rosenberg et al. 2000; Kaufmann et al. 2004), a more recent study (Hecht et al. 2006) comparing early intervention (<14 days) with later (2 weeks to 6 months) identified immunological benefit in both groups although enhanced outcome was only seen with earlier intervention.

It is also of interest to note that while viral testing is an important tool for the detection of an acute HIV infection, it does not serve as the stand alone diagnostic assay and the recommendations are that when acute HIV infection is diagnosed by a positive viral test (such as HIV RNA or p24 antigen) coupled with a negative HIV antibody test, a confirmatory HIV antibody test should be performed over the next 3 months to confirm seroconversion (DHHS and OARAC 2011). Thus, the ability to detect HIV specific antibodies, at the acute phase, by using the SMARTube could play an important role in providing immediate confirmation of HIV diagnosis by specific antibody assays.

6.7 A potential value of initiating treatment early

In 2007, it was stated that based on the measurements of decay of the HIV reservoir in patients who initiated antiviral therapy early in infection, the half-life of this latent viral reservoir was estimated to be 4.6 months. With this, it was projected that it will take up to 7.7 years of continuous therapy to completely eliminate latently infected resting CD4+ T cells in infected individuals who initiate antiviral therapy early in HIV infection (Chun et al. 2007). This and other studies have led to the initiation of the experimental Test and Treat programs.

6.8 Measured potential benefits of early treatment
6.8.1 Increase in life expectancy due to early treatment

Recently it has been estimated that an expanded HIV test and treat program in Washington DC will increase life expectancy of HIV-infected patients but will have a modest impact on HIV transmission over the next 5 years and is unlikely to halt the HIV epidemic (Walensky et al. 2010). In another paper (Bendavid et al. 2010) it was predicted that early treatment, when compared to the status quo, universal testing and treatment, was associated with a life expectancy gain of ~12.0 months of life, and ~35.3% fewer infections over a 10-year time horizon. Their results support the notion that universal testing and treatment could have significant mortality benefits. A recent estimate from South Africa suggest that ART may prolong life expectancy of infected individuals by 12.5 years (Walensky et al. 2009).

6.8.2 Reduction in community viral load

San Francisco Department of Public Health reported that as 'community viral load' (the amount of virus in the blood of all HIV-infected individuals tested in San Francisco) declined from 2005 to 2008 because of drug treatment and increased awareness, the number of new infections in the city also dropped (Das et al. 2010). Similar results were presented for a study of IDU in Vancouver (Wood et al. 2009). Recently it was reported that when treatment was expanded to IDU with HIV throughout British Columbia, new HIV diagnoses in that group dropped by around 50%.
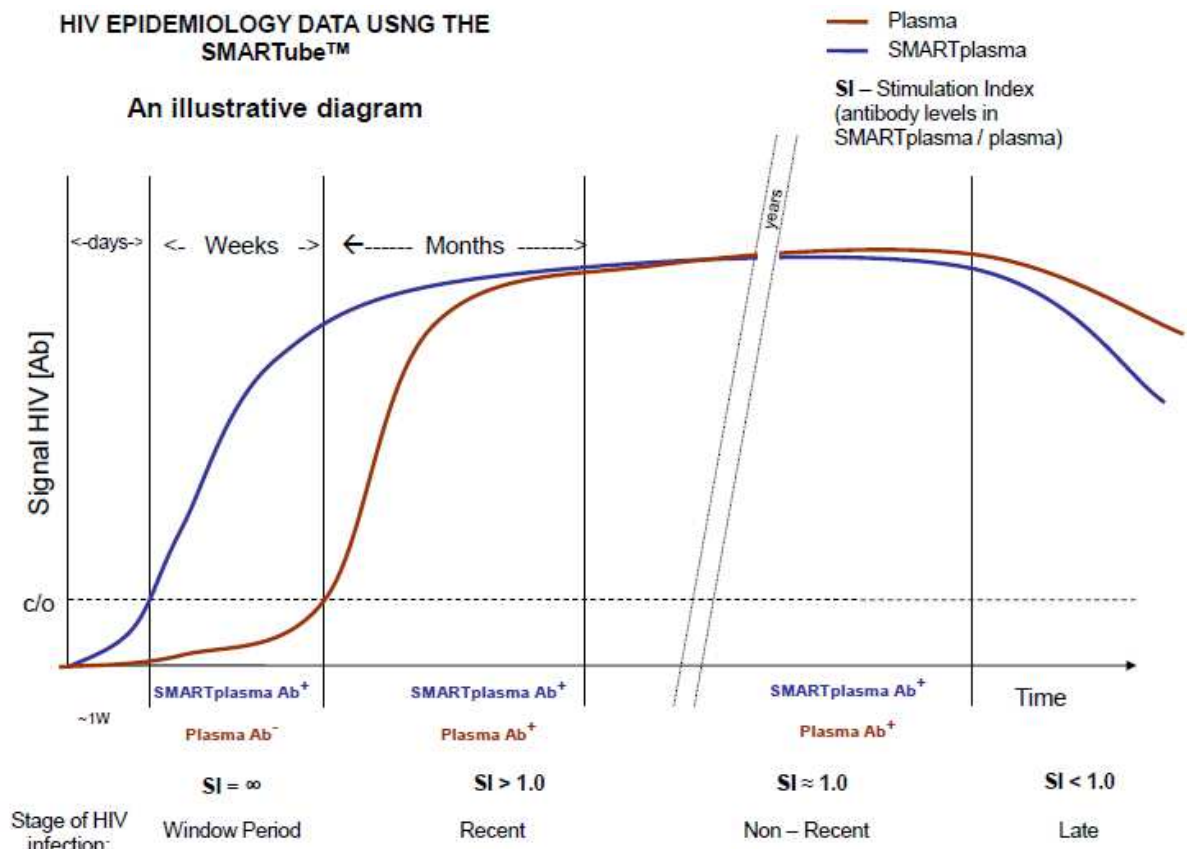

Fig. 6. Detecting very early (WP) HIV infection and differentiating recent from non-recent seropositive infections by using the SMARTube and comparing the levels of HIV antibodies in SMARTplasma versus plasma – An illustrative diagram

6.8.3 Reduced rate of transmission

Recently results were published (Granich et al. 2009) showing that test-and-treat program could eliminate HIV transmission, defined as an incidence below one case per 1,000 people per year, within a decade. This was supported by a report based on independent studies in Canada (Check Hayden). In the United States, treating HIV infection aggressively before symptoms appear could help to control the spread of the disease (Hamlyn 2010). These findings could have an effect on the WHO ARV treatment policies in the future.

The initial stage, acute HIV infection, has a short duration (measured in weeks to months), is difficult to diagnose, and is associated with high levels of viremia (Dieffenbach and Fauci 2009). The large amount of virus in most newly infected individuals renders them highly infectious to others (Pinkerton 2008). Thus these primary and early infections should be the ones that early treatment is focused on. The challenge is to detect the infection early, and also to be able to differentiate between recent and non recent infection once diagnosed. The SMARTube offers a unique tool to enable both detection of infection within a week and the differentiation of recent from non-recent infection.

7. What is the length of the seronegative WP?

7.1 Statistical estimation guiding public health institutions

The WP is a general HIV phenomenon, that is, antibodies are not produced against HIV within a week or so of infection. The current epidemiological-diagnostic WP is estimated to be 8-10 weeks (and >95% of the infected individuals will seroconvert within 3 months) (CMA 1995; CDC 2001; Branson et al. 2006; Workowski and Berman 2010). Thus, there is an underlying mechanism of initial specific immune suppression which would affect the length of the WP, and which varies pending genetic, environmental, and immunological background.

The length of the WP should not be confused with the length of the active viremia which precedes the Seroconversion (Fiebig et al. 2003). The estimated time "0" of that active viremia stage (Busch et al. 1995), has nothing to do with the time of infection. There has been reported cases where virus has been detected 10-14 days post infection due to blood or organ donation which was in the WP. However, the duration of the WP in the donor, as well as the duration of the WP in the more common routes of infection (e.g. sexual, IDU) and "natural doses" of inoculums (Hladik and McElrath 2008) are generally unknown and cannot be derived from the transfusion and transplantation cases.

7.2 Estimating the length of the WP using the SMARTube

Classically, as time of infection is mostly unknown, the time of seroconversion is calculated, based on the median between the last seronegative and the first seropositive sample. Since with the SMARTube detection of HIV specific antibodies can be achieved within days of infection, the time of infection can be better estimated (the median between the last SMARTplasma negative and SMARTplasma positive results for HIV specific antibodies). This, coupled with the time of seroconversion, it can offer a tool for calculating the length of the seronegative WP in a given population.

In some cases the time of infection can be statistically estimated in a population based study, when there is migration at a given time point from a low risk area and life style to a high risk area and life style. With the aid of the SMARTube, and given the defined, relatively short, time of exposure to HIV in that immigrant population, the length of the WP in such a

population was estimated (Novikov and Jehuda-Cohen 2009). The variables shown in table 4 include the number of infectious days (IP) within the 365-day stay in the refugee camp, defined here to range between 183-304 days. Furthermore, the time interval for performance of the blood tests ranged between 0-90 days (TP) of arrival in Israel. A window-period of 15 days, was assumed for any viral infection prior to humoral immune responses. These variables were run in calculations used to determine the WP length while assuming a ratio of 1, 50 or 90% of infections due to internal sexual contacts. All in all the length of the "mean" WP was estimated to be 160 days (>5 moths).

Fig. 7. The time line from infection to detection, and the contribution of the SMARTube, which enables detection during the window period, as soon as there are primed B cells in the blood.

Infectious period (IP)	Testing period (TP)	Calculated window period length (days)					
		Percent of internal contacts					
		1%	SD	50%	SD	90%	SD
183	30	119.5	20.2	110.2	19.9	80.3	17.5
183	90	148.4	20.4	139.2	20.5	111.5	17.9
243	30	154.1	26.7	141	26.1	102.1	24.5
243	90	182.3	27.5	167.8	28	132.6	24.2
304	30	188.1	33.8	171.9	34.4	122.6	29.2
304	90	215.6	34.4	199.4	34.9	153.9	30.1

Table 4. Calculation of HIV window period lengths based on SMARTplasma versus plasma HIV antibody results.

Some follow up studies were conducted testing individuals who tested seronegative in the initial screening. A total of 16 new infections were identified, all of which were SMARTplasma positive, only 5 of them were plasma positive, i.e. 11 of those 16 new infections, were SMARTplasma positive in a blood sample, prior to seroconversion. Of those 11 individuals who were identified during the seronegative WP, 9 were followed until seroconversion. All those 9 (100%) SMARTplasma positive, yet seronegative for HIV antibodies, seroconverted within 1 to 5 months from the time of first SMARTplasma positive, yet seronegative sample. From these studies it can be concluded that the SMARTube™ enables the detection of HIV antibodies (and thus HIV infection), weeks and months prior to seroconversion.

8. Conclusions

The seronegative WP is an important factor in diagnosing the HIV infection, understanding its immunological- pathological sequella, monitoring the transmission and spread of HIV, and controlling the HIV epidemic. An important key to all the above is being able to detect the infection within days, and thus study the earliest possible interactions between the virus and the immune system. This earliest possible detection needs to be independent of the state of viremia, the location of the virus in the tissues, and seroconversion. A method which enables the detection of the initial, early stage, HIV primed B cells, has been developed which could open a window of opportunity to understand the HIV infection better and thus overcome its challenges to man and society.

9. Acknowledgment

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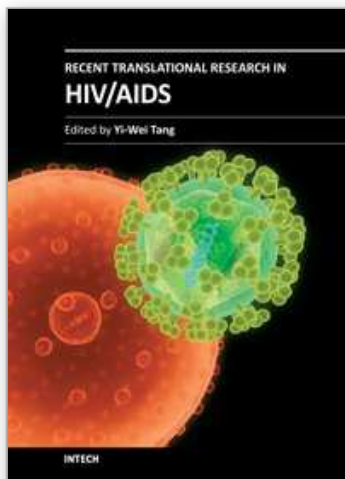
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Recent Translational Research in HIV/AIDS

Edited by Prof. Yi-Wei Tang

The collective efforts of HIV/AIDS research scientists from over 16 countries in the world are included in the book. This 27-chapter Open Access book well covers HIV/AIDS translational researches on pathogenesis, diagnosis, treatment, prevention, and also those beyond conventional fields. These are by no means inclusive, but they do offer a good foundation for the development of clinical patient care. The translational model forms the basis for progressing HIV/AIDS clinical research. When linked to the care of the patients, translational researches should result in a direct benefit for HIV/AIDS patients.

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