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"SILENT" CARRIERS OF HIV AND THE EPIDEMIOLOGY OF AIDS: A REVIEW

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ABSTRACT:

Notwithstanding that 30 years have elapsed since the discovery of the causative agent there is still no cure or vaccine for AIDS despite relentless efforts by researchers. This is complicated by the emergence of "silent" HIV carriers which are fueling the pandemic. "Silent" carriers are defined as seronegative, yet infected individuals who have never seroconverted. This should not be confused with late stage of AIDS, when infected individuals are severely immunocompromised, and the so-called "long window" period when antibodies are not detectable. Previous attempts to detect "silent" carriers have used a nucleic acid-dependent viral load test, Polymerase Chain Reaction (PCR) and Antigen Capture Assay (p24). The PCR that detects viral load as early as 2-4 weeks is costly and complicated; it is prone to false positives because of high sensitivity. The Antigen Capture Assay also has limitations of low sensitivity and false negative results. It is for these reasons that a new test called "stimmunology technique" -a SMARTube pre-analytical test is being advocated. This novel in-vitro enhancement technique enables accelerated pre-seroconversion confirmed diagnosis within a few days. The test is cheap, simple to perform and does not change the current algorithm for HIV antibody testing and diagnosis. This technique corroborates what WHO says; that the opportunity to control the epidemic lies in detecting the infection in its early stages and focusing prevention and treatment on them.

***KEYWORDS**: Impact, HIV, Silent carriers, AIDS Epidemiology*

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INTRODUCTION:

During my postdoctoral fellowship at the university college hospital in London, a fellow researcher on HIV/AIDS jokingly said it is about time we changed the meaning of the acronym "AIDS" to be "Acquired Inhuman Deficiency Syndrome". Everybody laughed. But it is no longer a laughing matter when you consider that AIDS has defied consistent efforts of researchers to produce either a cure or vaccine and the disease has robbed the world of some 25 million lives since its advent over 30 years ago and still decimating the sanctity of mankind! Such a disease is truly inhuman.

However, in his article titled "Berlin patient spreads hope for AIDS cure" in Washington Times in July 2012, Cory Brown [1] reported about the man believed to be the only patient completely cured of AIDS virus. He is Timothy Brown, known in medical circles as "The Berlin Patient". His physician, a German haematologist, Gero Hutter treated him with a revolutionary therapy - a stem cell bone marrow transplant from a donor who had natural immunity to HIV and leukaemia for which he was also diagnosed. Allers and colleagues had earlier reported this evidence of the cure of HIV infection [2].

There are several conflicting history and theories about the origin of AIDS. It would appear that scientists now believe that HIV probably transferred to humans in Africa between 1884 and 1924 [3]. Studies have shown that HIV first arose in Africa and spread from primates to humans early in the 20th century, most probably when humans came into contact with infected blood during a chimpanzee hunt [4] (The Hunter's theory). By testing stored blood samples, scientists have found direct evidence of a human being infected as far back as 1959 with a virus now classified as HIV [5]. In the 1970s African doctors saw a rise in opportunistic infections and wasting, while western scientists and practicing physicians were not yet aware of the growing epidemic. The origin of AIDS and HIV has puzzled scientists ever since the illness first came to light in the 1980s. At this juncture one can only rehearse what an erstwhile president of Zambia - Dr. Kenneth Kaunda said, "……..that we should get more concerned with where AIDS is going rather than where it is coming from".

On June 5, 1981 the United States Center for Disease Control and prevention (CDC) published an article in their Morbidity and Mortality Weekly Report describing cases of rare lung infection *Pneumocystis carinii* pneumonia in 5 young previously healthy gay men in Los Angeles [6]. It was then termed "gay plague". By the end of that

year there were a cumulative total of 270 reported cases from physicians across the United States of severe immune deficiency among homosexuals and 121 of those individuals have already died. We are now aware that the disease has spread to every corner of the globe and is the leading cause of infectious death worldwide. According to the WHO over 35 million people have been infected worldwide, 25 million dead since the pandemic, 2 million dying every year, 250,000 being children and that over 50 percent are women [7]. There are over 6000 infections daily and most of them are occurring in Sub-Saharan Africa [7]. Thus, Africa has been designated as the center point of the AIDS pandemic recording some 25 million of AIDS population. The AIDS epidemic not only affects the health of individuals, it impacts households, communities and the development and economic growth of nations [7]. The immune-pathogenesis of HIV infection is presented in the schematic diagram in Fig. 1.

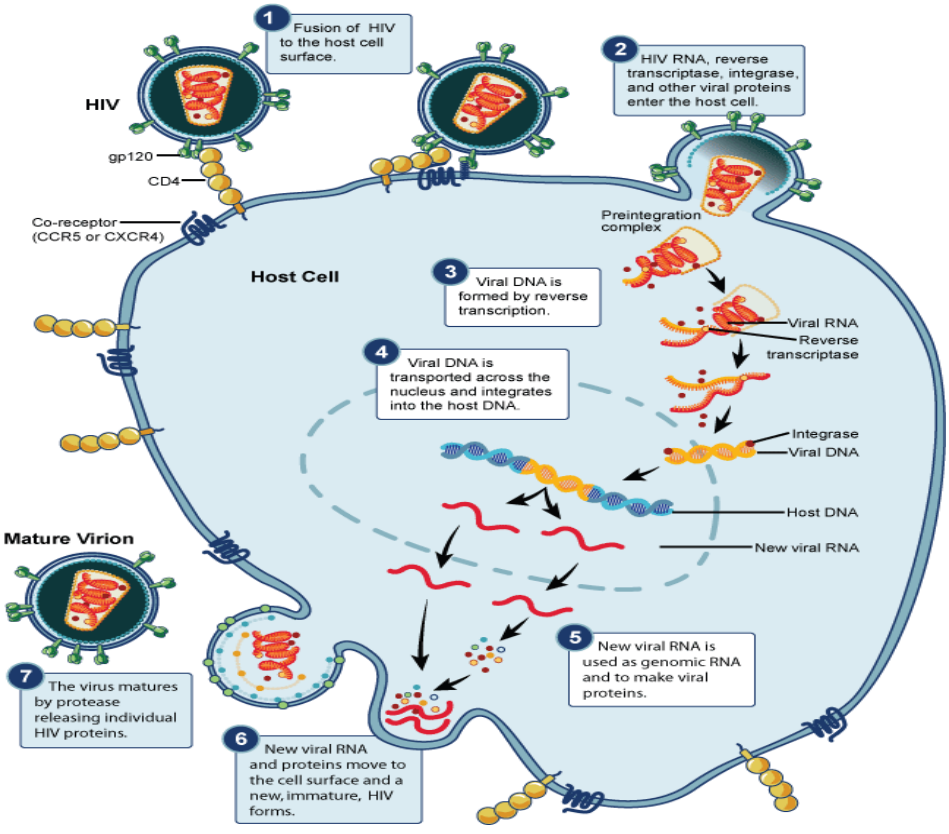


Fig. 1: Schematic diagram of the immune-pathogenesis of HIV infection (Adapted from National Institute of Allergy and Infectious Disease (NIAID) [8]

Transmission is through exposure to sexual bodily fluids, cerebrospinal fluid, sharing needles, tattooing or piercing with contaminated equipment from an infected individual, from mother to her unborn child during pregnancy or delivery [9]. Transmission through blood transfusion is rare as well as through anti-haemophilic factor VIII since blood is screened and factor VIII heated especially in developed countries [9].

The HIV targets CD4 immune cells and the nervous system. During the course of infection, patients experience gradual decline in CD4 cells which immunocompromises them to the development of life threatening opportunistic infections, (viral, fungal and protozoal), Tuberculosis, extreme weight loss, cancers (such as Kaposi sarcoma) and dementia [10]. However, patients consistently taking anti-retroviral medication are likely to control the infection and not progress to full blown AIDS [11].

The predominant virus is HIV-1 and the relatively uncommon type is HIV-2 which is concentrated in West Africa and rarely found elsewhere. The strains of HIV-1 remained groups M, N, and O until Plantier et al. identified a new strain in a Cameroonian woman in 2009 [12], which they designated group P. Within group M of HIV-1 there is a total of 9 genetic subtypes or clades identified and are designated A, B, C, D, F, G, H, J, K, within which are Circulating Recombinant Forms (CRFs) which developed as a result of viral sexual reproduction. Examples of such are CRF A/B and CRF A/E because it could have resulted from a hybridization between subtype A and some other “parent”, subtype E, although no one has yet isolated subtype E in pure form [13]. In addition, 10 genetic subtypes of HIV-2 have been detected worldwide [14].

The relevance of HIV subtypes is reflected on seroepidemiology, transmissibility, therapy and vaccine production [15]. Differences in transmission and disease progression occur between these subtypes and CRFs. For example, subtype D is shown to be more virulent and accelerates development of AIDS more than subtype A, because it is more effective in binding to immune cells. Subtype B is found to be spread mostly by homosexual contact and intravenous drug use while subtype C and CRF A/E fuel heterosexual epidemic [12] Are more subtypes likely to occur? It is almost certain that new HIV subtypes as well as CRFs will be described in the future as virus recombination continues to occur [16]. The current subtypes and CRFs may also continue to spread to new geographical areas as the global epidemic continues [13].

The search for a cure for HIV began as soon as the virus was identified in 1983 [17]. HIV is probably the most studied virus in the history of

scientific research. Scientists have detailed knowledge of the virus replicative cycle, genes and proteins and also understand their functions. One of the problems with finding a cure is that the virus can hide in cells throughout the body and also hide in areas that are difficult for drug to reach such as the brain. These infected cells that persist in the body are being studied to determine how they can be stimulated to produce virus and /or be targeted for clearance from the body with new drugs that are being developed [16]. Fortunately nearly all subtypes identified so far are responsive to anti-retroviral drug. However, some studies have found variation in the ways different subtypes and CRFs respond to anti-retrovirals [12]. Unfortunately HIV cannot be cured, with the exception of the case of the Berlin Patient mentioned earlier. Nevertheless the Highly Active Antiretroviral Therapy (HAART), which is a poly-therapy consisting of a combination of several antiretroviral drugs, does delay or potentially completely stops disease progression, thus allowing an infected individual to live longer healthy life. This is because antiretroviral drugs can lower the viral load and raise the CD4 count [10]. However, it is a myth that people on anti-retroviral medication cannot transmit HIV to their sexual partners. Since there is no effective vaccine for HIV currently, post exposure prophylaxis (PrEP) is being advocated for HIV prevention in high risk individuals, especially those with multiple sexual partners and those at occupational risk. Currently gene therapy is been viewed as having the potential to engineer HIV control by introducing cells resistant to the virus [18]. A clinical trial using gene-editing techniques successfully targeted and destroyed a gene in the immune system of 12 people living with HIV, increasing their resistance to the virus [18]. However, because of the invasive nature of stem cell therapy, it is not viable for the majority of people living with HIV since the body is likely to attack the donor cells as a sequel of transplantation [18].

The search for effective vaccine for HIV is still in progress and remains an active area of research. When presenting lectures on HIV/AIDS, as a United Nations Peer Educator on HIV/AIDS, I emphasize to participants that, currently one of the most effective alternatives to “vaccine” is public enlightenment. If there was a microbicide for HIV infection, as we have in bacterial diseases, it would have been applied per vaginam or per rectum to prevent the virus being passed on sexually and may kill the HIV or inhibit its replication. Development of HIV/AIDS vaccine is affected by a range of factors including continuous genetic variability of HIV, through mutation and recombination, which poses a

scientific challenge [19]. The vaccine scientists have to discover aspects of the virus that are consistent enough to provoke an effective immune response against multiple HIV variants and have the full understanding of the correlates of immunity. Since some potential vaccines work only against particular subtypes, one can suggest that prevailing subtypes in any geographical area should always be included in the vaccine. Such was the case in Thailand when an experimental vaccine was modified following a report of molecular epidemiologists that the predominant subtype B had been replaced with another subtype E in a population of injecting drug users among whom the trial was to be conducted [15].

The other problem is the wide variety of human populations who need protection and differ in their genetic make-up, including human leukocyte antigen diversity (HLA) and their routes of exposure to HIV. To add to these problems is the occurrence of HIV super infection which indicates that an immune response triggered by a vaccine to prevent infection by one strain may not protect against all other strains of HIV. This is one of the problems being addressed by the Council of Global HIV Vaccine Enterprises as well as Homsy and colleagues [20-21].

Perhaps a saving grace, while scientists are still battling with the problems of developing an effective HIV vaccine, is the Passive Immunotherapy (PIT) developed by Abraham Karpas [22]. He noted that infected individuals develop an early vigorous antibody response to infection which often lasts for several years. This led him to suggest that blood plasma collected from these “healthy” HIV infected individuals which contains antibodies that can neutralize and kill viruses including HIV could then be given to AIDS patients. Immunity to the virus in form of antibodies, is passively transferred from the “healthy” HIV individuals to the sick AIDS patients, hence the term passive immunization [22]. Results published as far back as 1988 suggest that passive immunotherapy is currently the best form of treatment for people with HIV disease, and therefore raises the prospect of reducing the spread of HIV in the population [22].

“Silent” HIV Carriers:

Perhaps there is no better way to introduce this section on HIV silent carries than paraphrase, what AVERT stated: that the spread of AIDS could quite conceivably have been induced by a combination of many different events [13]. Whether through injections, travels, wars, colonial practices or genetic engineering, the realities of the 20th century have undoubtedly had a major role to play. Nevertheless, a more pressing concern for scientists today should not

be how AIDS epidemic originated, but how those infected with HIV can be treated; how the future spread can be prevented and how the world can change to ensure that a similar pandemic never occurs again [13].

It is on this premise that we have to look at those seronegative, yet infected individuals who have never seroconverted and yet spreading the disease. These individuals are called “Silent” HIV Carriers. These individuals should not be confused with those at late stage of AIDS, when they are so immunocompromised that they are unable to mount an immune response to produce antibodies or those in the “long window” period or those with genetic “defect” to produce HIV-specific antibodies.

Research has shown that the widely assumed “latent period” (Window period) of HIV infection was utterly fictitious. Rather a “titanic” struggle erupts, perhaps from the time the virus first gains a foothold. In the struggle the body’s CD4+ T immune cells respond furiously, die daily in billions, are prodigiously replaced, but ultimately overwhelmed by fulminating HIV replication [23]. This may mean that even in the window period antibodies can still be produced if the cells are stimulated.

Why the concern about silent carriers [24]?

- They can transmit HIV and fuel the pandemic, thus negating all the efforts so far in containing the spread.
- Each unknowing HIV carrier can infect at least 50 or more individuals each year according to the hypothesis proposed by Frerichs, who extrapolates that about 12-14 million silent carriers roam the world.
- False negative individuals tend not to be retested 3 months later, which is the recommended practice; thus they may contribute to the spread of HIV.

As an example, a patient was assumed to be a silent carrier because she was consistently HIV seronegative; although she was seronegative until she died, she was not a silent carrier. Because we did not know what to call it we used the term AIDS-related illness [25] in line with what Onwubalili and colleagues had earlier described in a group of patients who also died from AIDS-related illnesses [26]. We then advised surgeons to be aware of such patients and endeavour to screen their patients before surgery [27]. These finding made me more cautious, during my United Nations years, working as HIV/AIDS Specialist and as community-based physician. It is possible that some of the high risk seronegative patients that presented could have been HIV silent carriers.

Knowledge of existing HIV strains in a particular geographic area is as important as knowledge of endemic diseases that can mimic HIV infection or affect HIV tests, such as chronic parasitaemia, for example due to malaria [28].

Three groups of basic HIV tests are:

- Antibody tests: Enzyme-Linked Immunosorbent Assay (ELISA) and Western Blot (WB) which is a confirmatory test.
- Antigen test also known as p24.
- Nucleic Acid Test: Polymerase Chain Reaction (PCR) an RNA or DNA-dependent Viral Load Assay.

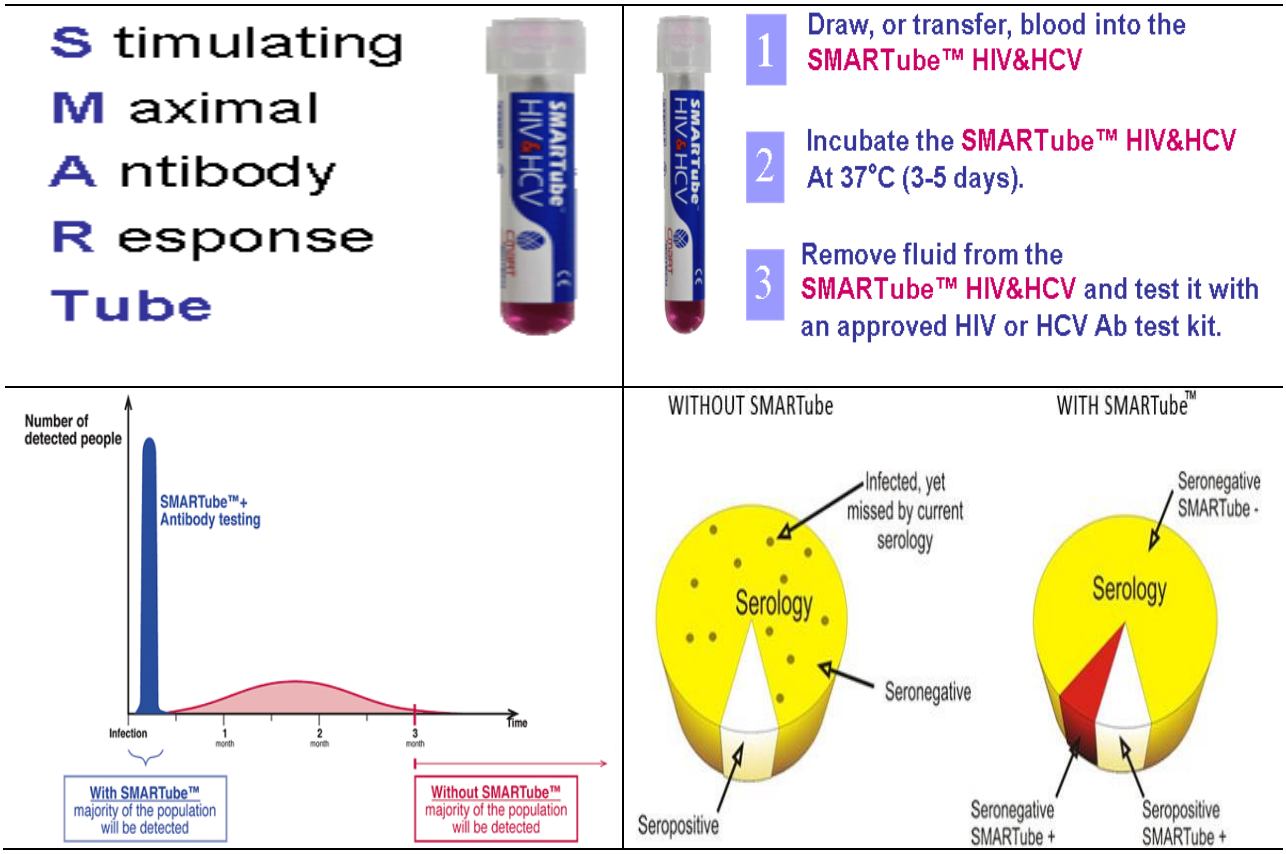
If both ELISA and WB are positive the chances are that in more than 99% of cases the patient is infected. It is critical that pregnant women, who seem to have a long window period, be tested because medications are very effective in reducing transmission from mother to child. A pregnant woman testing false-negative for HIV will not be offered anti-retroviral treatment which could have saved her baby. HIV tests may miss some infections, resulting in false negative results. This often occurs soon after infection when antibodies are just starting to form and are at a level too low to be detected. Other tests that have been used in HIV serology include the Particle Agglutination Test for HIV antibody developed by Fujirebio Inc. of Japan, the Karpas Cell Test and the HLA Testing developed in London Hospital Medical College [29]. Earlier attempts to detect HIV silent carriers have employed the PCR as well as the antigen capture test called p24. These tests have the disadvantages of either cost or technical complexity in performing them as in PCR or low sensitivity as well as false negative results which both of them share. These tests detect infections only when they have lasted several weeks. In the case of PCR false positive results have been reported [30] or failure to detect HIV [31] in a study involving 92 female prostitutes and their heterosexual partners of infected individuals.

The Stimmunology Test was developed to help resolve the problems of false negative results that tend to complicate the epidemiology of AIDS [32]. Stimmunology technique is an antibody enhancement pre-analytical procedure using immune potentiating agents. Stimmunology technique uses the Stimulating Maximal Antibody Response Tube- (SMART) Test. An overview of the procedure is illustrated in the diagram shown in Fig. 2 (adopted from Biotech Inc.).

SMARTube is not a diagnostic test; it is an in-vitro pre-test enhancement of antibody output, by stimulated B lymphocytes that were primed by HIV in the infected person, leading to early

detection of HIV infection even prior to seroconversion. Some of the advantages of Stimmunology are: Promotes early diagnosis (3-5 days); relatively cheap; Simple to perform; Simultaneous detection of both HIV and Hepatitis virus (HCV); Overcomes non-responsiveness due to immune-suppression; Does not change the current algorithm of antibody tests and diagnostics that are in use.

Fig. 2: The Stimmunology technique: Schematic representation of the procedure using the **S**timulating **M**aximal **A**ntibody **R**esponse **T**ube- (SMART) Test (Adopted from Biotech Inc. [33])



Although observation about the potential impact of HIV silent carriers was made over 20 years ago, the initial research to highlight the possible impact of silent retroviral infection carriers on the epidemiology of AIDS were conducted in nonhuman primates and later in humans [34-47].

The major results obtained from these studies indicated that when whole blood from seronegative subjects was stimulated most of them produced HIV-specific antibodies. One of such studies was conducted by Mumo et al. [41]. They reported that pre-treatment of whole blood sample in SMARTube containing immune potentiating agents promoted the synthesis and release of antibodies against HIV-1 prior to their detection in corresponding plasma samples in a group of donors who would have been otherwise classified as HIV-1 seronegative (Figure 2). This is critical for saving lives not only through a safer blood supply but also by detection of HIV infection among pregnant women who seem to have a very long window period. More so, since a pregnant woman testing negative for HIV, as correctly stated by Jehuda-Cohen, may not be given any anti-retroviral therapy that could have saved her baby [41].

In their study, using polyclonal B-cell activation test (PBA-T) and PCR among wives of seropositive HIV carriers in Ethiopian community in Israel, Jehuda-Cohen and colleagues reported that 8 of the 12 silent carriers studied were discordant for serum antibodies, yet 5 of the 8 seronegatives (66%) were found to be PBA-T and PCR positive for HIV [42]. Aiuti and coworkers, investigating 65 HIV silent carriers showed that 12 (18%) of them had HIV-1 proviral sequences by PCR. They concluded that prolonged seronegative individuals can transmit HIV through their body fluids [43].

DISCUSSION:

Medical science has taught us that anything hidden in the body is a threat to humanity. When cancer cells begin to grow in the body, they are not easily noticeable until you screen and identify them, after which management can commence. In the same way, most individuals do not know that they have essential hypertension, whose symptoms are silent. They may only be informed that their blood pressure is high during a regular medical check-up. Silent HIV infection is not an exception.

Several studies have indicated unambiguously that silent HIV carriers fuel the epidemic of AIDS. Ralph Frerichs, [24] in his extrapolation, contends that about 12-14 million silent HIV carriers roam the world. He indicated that “anonymous testing and absolute confidentiality,

as promoted by many public health officials, are self-defeating, making winners of the virus and losers of the people".

The impact of HIV silent carriers on the epidemiology of AIDS cannot be overemphasized. During the 6th International AIDS Society conference in Rome, Jehuda-Cohen [48] discussed the results of an independent multicenter study in five countries involving 5000 high-risk and 3000 low-risk silent carriers. The researchers were able to show that there were no false positive results in the low-risk population thus providing improved specificity, and that the use of SMARTube can identify early HIV infection, as shown by increased detection over regular plasma from infected person, thus improving sensitivity.

The study also showed that epidemiologically, the SMARTube Stimulation Index (ratio of antibodies in SMART plasma/plasma) indicates a low false recent rate, resulting in excellent specificity which is essential for determination of incidence (rate in which the epidemic is spreading). This they contended is the key to public health efforts in carrying out epidemiological studies to determine where high rate of new infections are occurring and for monitoring the efficacy of preventive programmes.

In conclusion, it was stated that both WHO and CDC are working towards a wide acceptance of new approaches, such as SMARTube, to combat the spread of HIV such as early and complete detection of HIV infections to facilitate the concept of "test and treat" which they support as a major advance in helping to curb the spread of HIV infection. It is therefore being advocated that strategies for preventing potential transmission from HIV-1 infected but seronegative individuals be addressed. Common sense dictates that failure to recognize and implement such strategies condones significant threat to the spread of HIV-1 among the world's most affected population. HIV antibody testing is the gold standard for HIV diagnosis. While it is quite effective for general use, conventional antibody-based testing for HIV infection does not allow for a thorough evaluation of a population's epidemic state since it excludes individuals within the "window period" which is estimated at 3 months for 95% of infected individuals [49, 50].

Thus, it is common practice that people at risk for HIV infection, who tested negative, are usually requested to come back for re-test 2-3 months later. Majority of them do not come back because they do not appreciate the need to do so. We

have to shift the paradigm of laboratory research from mere laboratory curiosities to result-oriented, problem-solving and cost-beneficial exercise. It is for these reasons that I recommend the simple cost-effective and reliable stimulating antibody assay - Stimmunology SMARTube assay for use in resource-poor setting to increase blood supply safety and quantity. Incorporating Stimmunology into basic blood bank testing and diagnostic protocols can also decrease preventable disease transmission.

" Life affords no higher pleasure than that of surmounting difficulties, passing from one step of success to another, forming new wishes and seeing them gratified" - Samuel Johnson

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