HIV Infection, Screening and Confirmatory Testing

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Abstract

Advances in human immunodeficiency virus (HIV) treatment have changed the natural history of HIV and improved the quality of infected patients. Post exposure and vertical transmission prophylaxis do not change the main features of HIV prevention. This article reviews the common screening and confirmatory tests for HIV along with statistical information related to HIV in Hong Kong.

Introduction

In Hong Kong, the first case of HIV infection was reported in 1984. Since then, the number of reported cases was growing steadily. In 2005, a total of 313 HIV cases were reported to the Department of Health (DH). This figure represents a 17 per cent increment as compared to 268 in the preceding year. This is the highest annual number recorded.

Reviewing disease transmission, sexual transmission has continued to be the major mode of spreading, which accounted for 64 per cent (201 cases) of the newly reported cases. For the remaining contacts, intravenous drug use accounted for 8% (25 cases), blood/blood product transfusion 1.3% (4 cases), and perinatal infection 0.6% (2 cases). The remaining 25.9% (81 cases) had undetermined routes of transmission.

In 2005, the number of AIDS reported was 64, a stable figure as compared to the previous 10 years. To date, it is estimated that over 3,000 persons are living with HIV/AIDS in Hong Kong. High HIV infected rates in neighboring cities, extensive human mobility across border, and the practice of risk sexual behaviors are some of the important factors that may predispose to a upsurge of the epidemic.[2]

HIV INFECTION

Acquired Immuno-Deficiency Syndrome (AIDS) is a transmissible disease of the immune system caused by the human immunodeficiency virus (HIV). HIV slowly attacks and destroys the immune system of the host leaving an individual vulnerable to a variety of other infections as well as certain malignancies that eventually cause death. AIDS is the final stage of HIV infection.

The Emergence of AIDS

AIDS was first reported in 1981 in USA primarily in New York and California. Initially most cases of AIDS in the United States were diagnosed in homosexual males, who contracted the virus primarily through sexual contact, and in intravenous drug users, who became infected mainly by sharing contaminated hypodermic needles. In 1983 French and American researchers isolated the causative agent, HIV. By 1985, serological tests were tired developed to detect the virus.[3] The spreading of HIV (AIDS) was significant in the 1980s, particularly in Africa, where the disease may have originated. The common modes of HIV spreading include international travel, changing sexual partners, and intravenous drug use etc. According to the United Nations 2004 Report on worldwide AIDS, some 38 million people are living with HIV, approximately 5 million people become infected annually, and about 3 million people died each year since 1981.[4] Some 20 million people were already dead because of the disease

Transmission

HIV can be transmitted through direct contact of body fluids, such as blood, semen, genital secretions, and breast milk to an uninfected person. The virus initially enters the body through the mucosal lining such as the urogenital tract and mouth. Prior to the development of screening procedures for HIV, and heat treatment blood products, transmission can be occurred through the contaminated blood products. Today the risk of contracting HIV from blood transfusion is extremely small. In rare cases transmission to health care worker may occur by accidental stick with an infected needle of a HIV carrier. The

virus also can be transmitted across the placenta or through the breast milk from mother to infant. The virus by itself is fragile and cannot survive long enough to cause infection outside of the body. HIV will not be transmitted by coughing, sneezing or casual contact (e.g. shaking hands). Strictly spreading, direct transfer of body fluid containing HIV is required for the actual transmission. Other sexually transmitted diseases, such as syphilis, genital herpes, gonorrhea, and chlamydia, increase the risk of contracting HIV through sexual contact, probably through the primary sites of infections.[2]

Life Cycle of HIV

The main target of HIV infection is helper T lymphocytes, or helper T cells, a special class of white blood cells of the immune system. Helper T cells are also known as CD4+ T cells because they have on their surface a protein called CD4. It plays a central role in normal immune responses by producing factors that activate virtually all the other immune system cells. These include B-lymphocytes, which produce antibodies needed to fight against infection; cyto-toxic T lymphocytes, which kill cells infected by viruses; and macrophages and other effectors cells, which attack invading pathogens. AIDS is the results of the loss of most Helper T-cells in our body.[4]

HIV is an retrovirus that consists of genetic material in the form of RNA surrounded by a lipoprotein envelope. HIV cannot replicated but relies on the infected host cells to produce new viral particles. HIV infects the helper T cells by means of a protein embedded envelope called gp120. The gp120 protein binds to a CD4 molecule on the surface of the helper T cells that triggers a sequence of events that allow HIV genetic information to get into the host cells. The actual entry of HIV into the host cells requires the participation of a set of cell surface proteins that normally serve as receptors for chemokines (i.e. hormone-like mediators that attract immune system cells to particular sites in body). It appears that the binding of gp120 to CD4 exposes a region of gp120 that interacts with the chemokine receptors. This interaction triggers a conformational change that exposes a region of the viral envelope protein gp41, which inserts itself into the membrane of the host cell that bridges the viral envelope and host cell membrane. An additional conformational change in gp41 pulls the two membranes together, This fusion allows the viral genetic material entering into the host cell.[3]

Once the helper T was infected, HIV copies its RNA into a double-stranded DNA of the host cells by means of the viral enzyme reverse transcriptase. This process is called " reverse transcription " because it violates the usual way in which genetic information is transcribed. Since the enzyme reverse transcriptas lacks the "proof-reading" function that most DNA synthesizing, This enzymes have, many mutations arise as virus replicates, further hinder the ability of the immune system to combat the virus. These mutations allow the virus to evolve very rapidly, approximately one million times faster than the human genome. This rapid evolution allows the virus to escape from antiviral immune responses and antiretroviral drugs.

Under appropriate conditions, viral infected DNA of helper T cells are transcribed into viral RNA molecules. Some viral RNA molecules are incorporated into new virus particles, while others are used as messenger RNA for the production of new viral proteins. Viral proteins assemble at the plasma membrane together with the genomic viral RNA to form a new virus particle that buds from the surface of the infected cells, taking with it some of the host cell membrane that serves as the viral envelope. Embedded in this envelope are the gp120/gp41 complexes that allow attachment of the helper T cells in the next round of infection. Most infected cells die quickly (in about one day). The numbers of helper T cells that are lost through direct infection or other mechanisms exceeds the number of new cells produced by the immune system, eventually resulting in a decline in the number of helper T cells. Physicians follow the course of the disease by determining the number of helper T cells (CD4+ cells) in the blood. This measurement provides a good indication of the status of the immune system. Physicians also measure the amount of virus in the bloodstream i.e. the viral load that provides an indication of how fast the virus is replicating and destroying helper T cells.[4]

Because of the high rate at which the genetic material of HIV mutates, the virus in each infected individual is slightly different. Genetic variants of HIV have been categorized into several major subtypes, which have different geographical distributions.

Course of infection

The course of HIV infection involves three stages: primary HIV infection, the asymptomatic phase, and finally AIDS. During the first stage, the transmitted HIV replicates rapidly, Some people may experience an acute flu-like illness that usually persists for one to two weeks. During this time a variety of symptoms may occur, such as fever, enlarged lymph nodes, sore throat, muscle and joint pain, rash, and malaise. Standard HIV testings which measure antibodies to the virus, are initially negative since HIV antibodies generally do not reach detectable levels in the blood until a few weeks after the onset of the acute illness. As the immune response to the virus develops, the level of HIV in the blood decreases.[3]

The second phase of HIV infection, the asymptomatic period, can last up to an average of 10 years with during this period.

With the virus continues to replicate a slow decrease in CD4 count (the number of helper T cells). When the CD4 count falls to about 200 cells per microlitre of blood , patients begin to experience opportunistic infections i.e., infections that arise only in individuals with a defective immune system. This is AIDS, the final stage of HIV infection. The most common opportunistic infections are Pneumocystis carinii pneumonia, tuberculosis, Mycobacterium avium infection, herpes simplex infection, bacterial pneumonia, toxoplasmosis, and cytomegalovirus infection. In addition, patients can develop dementia and certain cancers, including Kaposi sarcoma and lymphomas. Death ultimately results from the relentless attack of opportunistic pathogens or from the body's inability to fight off malignancies. A small proportion of individual infected with HIV has survived longer than 10 years without developing AIDS. These such individuals many mount a more vigorous immune response to the virus or they are infected with a weakened strain of the virus.[4]

HIV Screening

An arsenal of laboratory methods available to screen blood, diagnose infection, and 50 monitor disease progression in individuals infected by HIV. These tests can be classified into (a) detect antibody, (b) identify antigen, (c) detect monitor viral nucleic acids, and (d) provide an estimate of T lymphocyte numbers (cell pheno-typing). The focus of this on antibody detection, the most widely used and most effective way to identify HIV infection at present.

Tests to detect antibody to HIV can be further classified as: (a) screening assays, which are designed to detect all infected individuals, or (b) confirmatory (supplemental) assays, which are designed to identify individuals who are not infected but who have reactive screening test results. Accordingly, screening tests possess a high degree of sensitivity, whereas confirmatory assays have a high specificity. Tests with high sensitivity produce few false-negative results, whereas tests with high specificity produce few false-positive results. These classes of assays, performed in tandem, produce results that are highly accurate, reliable, and appropriate to protect the blood supply or assist in the diagnosis of HIV infection.

Technical errors do occur, however, and there are biologic factors that can limit the accuracy of HIV tests. Therefore, along with the testing process, there is the requirement for an extraordinary and dedicated quality assurance program.[5] Regardless of the results, because laboratory tests are not perfect, they are meant to be a supplement for clinical diagnosis.

Early Detection and the Window Period

Specific antibody to HIV is produced shortly after infection, but the exact time depends on several factors, including host and viral characteristics. Importantly, antibody may be present at low levels during early infection but not at the detection limit of some assays. Using the early generation tests, antibody could be detected in most individuals by 6 to 12 weeks after infection. Newer generation assays, including the third-generation antigen sandwich assays, can detect antibody at about 3 to 4 weeks after infection.[6]

Tests to Screen for HIV Infection

For the general laboratory diagnosis of HIV, the mere presence of specific antibodies signals that infection has occurred. For specific diagnosis, however, detection depends on the use of tests that are effective in identifying HIV antibodies, and not antibodies directed to other infectious agents that may be antigenically similar. Antigens used in HIV diagnostic tests must be appropriately specific, and are usually purified antigens from viral lysates, or antigens produced through recombinant or synthetic peptide technology. The use of such antigens allows HIV screening tests to possess both sensitivity (to detect infection) and specificity (to detect non-infection).

Regardless of the particular screening test used, serum or plasma samples are first (screened) using a test of choice with high sensitivity, such as a ELISA, "rapid test," or "simple method" (described below). ELISA is the screening method used most commonly, with the other two methodologies offer more rapid results with simpler procedures applicable for use in point of care testing and developing countries. With the advent of new therapies to treat HIV infection and the recommendation to institute therapy immediately (within 2 hours) after exposure.[7] Rapid assays may be the most appropriate to test the source patient after exposure(e.g., needle stick injuries).

Enzyme-linked Immunosorbent Assays/ Enzyme Immunoassays (ELISA/EIA)

ELISA are the most commonly used tests to screen for HIV infection because of their relatively simple methodology, inherent high sensitivity, and suitability for testing large numbers of samples, particularly in blood testing centers. More than 40 different ELISA test kits are currently available, but only about 10 are licensed by FDA for use in the United States.[6]

A common feature of all varieties of ELISA is the use of enzyme conjugates that bind to specific HIV antibody, and

substrates / chromogens that produce color in a reaction catalyzed by the bound enzyme conjugate. The most popular ELISA involves an indirect method in which HIV antigen is to the wells of a 96-well microtiter plate, or to a macroscopic bead that is subsequently placed in a well of a plate. Antibody in the sample is allowed to react with the antigen-coated solid support, usually for 30 minutes at 37 °C or 40 °C.[8] After a washing step to remove unbound serum components, conjugate (an anti-human immunoglobulin with a bound enzyme)is added a binds to the specific antibody that is attached to the antigens on the solid phase. Following another wash, an appropriate substrate is added for color development that is quantified by a spectrophotometer by measuring me to specific HIV antibody concentration in the sample. A mathematical calculation, usually based on the OD of the negative controls multiplied by a factor, produces a cutoff value (CO) on which the OD of the sample is compared to determine the antibody status; samples with OD/CO values greater than 1.0 (in an indirect ELISA) are considered as antibody reactive (positive).

Sensitive/Less-Sensitive ("Detuned") Assays

During acute HIV infection, prior to the appearance of antibody (window period or pre-seroconversion), HIV infection can only be confirmed by the demonstration of circulating p24 antigen, or the presence of viral RNA or DNA. Although highly sensitive antibody assays exist to detect very low levels of HIV antibody in blood, the window period prior to appearance of antibody can rarely be shortened to less than 3 weeks. Once antibody has appeared, titers progressively increase during 3-5 months until levels peak, at which time they remain fairly constant throughout the remainder of infection. Antibodies during early infection are usually of low avidity, but avidity increases as infection progresses. Therefore, HIV infection can be divided into categories of recent or established infection, depending on the quantity of antibody present or their avidities. These parameters can be exploited as tools in order to estimate the relative time that HIV infection occurred.[9]

For example, if antibody titers or antibody avidity is low, it is likely that infection occurred within the past 4 months; conversely, high-titer or high-avidity antibodies signal an established infection that has been present for longer than 4 months. In contrast to the prevalence of HIV infection; i.e. the number of persons infected, the incidence of HIV infection is defined as the change in prevalence of infection over time; i.e. the number of new infections occurring. Incidence estimates are often calculated to testing a cohort of individuals at two different time periods and observing the number of new infections within. This strategy is in fact difficult due to the need of locating individuals for follow-up testing. However, incidence estimates are important, not only for determining specific populations where educational endeavors can have the most benefit or where changes in infection patterns are occurring, but also to target these populations for therapeutic intervention or vaccines.[9]

New, laboratory-based, strategies have been devised to distinguish recently infected individuals from those with established infection. They are based on the concepts of antibody titer or antibody avidity, and modifications to the procedures of conventional ELISA or rapid assays have been performed to allow discrimination of antibody titer or antibody avidity. These modified assays have been called "detuned assays" or "sensitive/less sensitive assays (S/LS)".[9]

Fourth Generation Assays for the Simultaneous Detection of HIV Antigen and Antibody

By using the earlier generations of assays, Antibody can usually be detected in a majority of individuals within six to twelve weeks after infection. Antibodies can be detected within three to four weeks using the newer third-generation antigen sandwich assays.[6] The window period can be shortened to about two weeks by using p24 antigen assays or reduced to one week with the implementation of nucleic acid detection assays.[10] Consequently, the window period between infection and detection of infection may be less than two weeks if a comprehensive testing approach is utilized. The detection of p24 antigen by ELISA is a simple and cost effective technique to demonstrate viral capsid (core) p24 protein in blood during acute infection. In order to maximize the detection of all infected individuals, including those in early stage of infection, antibody, antigen and viral RNA tests should be used. However, viral RNA tests are expensive, time consuming, and are not commercially available in many laboratories. Laboratories that possess ELISA capability can increase their capacity to detect most of infections by testing for both HIV antibody and p24 antigen. During the late 1990s, assays in an ELISA format were develop that have the capability to detect both HIV antibody and HIV p24 antigen simultaneously, thereby eliminating the need to perform separate assays.[12]

The benefits of testing for both antibody and antigen are justifiable due to the need to identify individuals with both established and early HIV infection not only for the blood donor population, but also in some clinical applications. Early detection of infection via antigen testing promotes the prompt referral of infected individuals for the initiation of treatment, counseling, and preventive interventions to reduce the risk of transmission.

Rapid Tests

Rapid assays for detecting specific HIV antibody were developed in the late 1980s, and are defined as tests that can be performed in less than 30 minutes. These tests gained popularity in the early 1990s, As the technology became refined, it proved to be as accurate as ELISA when performed carefully by experienced personnel. Technical errors are common with these assays because users become careless with these simple procedures. For examples pipettes are not always held in a vertical position that resulting in an incorrect delivery of reagent volumes. Cause laboratory workers who attempt to test multiple samples simultaneously, inaccuracies in the timing of steps.[8]

Rapid HIV assays are accurate when performed correctly. They have wide utility in a number of testing situations. Application sites include: emergency rooms, physician's offices, point of care testing, autopsy rooms, funeral homes, small blood banks, and for stat HIV testing (where immediate treatment is recommended for exposures). Rapid HIV assays have proven particularly useful for testing pregnant women in labor who have not received prenatal care (i.e., their HIV status is unknown).[13]

One class of rapid tests is the "dot blot" or immunoblot" they produce a well-circumscribed colored dot on the solid phase surface if the test is positive. Most of these rapid assays now incorporate a built-in control indicating that test was performed correctly. This control is an anti-human immunoglobulin that binds any immunoglobulin in the sample and produces a separate indicator when all reagents are added appropriately. In addition, several varieties are available that include two "dots", which allow the differentiation of HIV-1 and HIV-2 infection. The procedures for dot-blot assays are similar regardless of the exact format of the test. Most require drop-wise additions of reagents in the following sequence: buffer, sample, wash buffer, conjugate, wash buffer, substrate, and stop solution. Some assays substitute an IgG binding dye (protein A gold reagent) for the anti-immunoglobulin conjugate, thereby decreasing the procedure by one step.[14]

Simple Tests

This type of HIV test requies greater than 30 minutes but has procedures that can be performed easily without instrumentation. Within this class of tests are agglutination assays in which antigen-coated particles (red blood cells, latex particles, or gelatin particles) are allowed to react with serum antibodies to form visible clumping (agglutination). If RBC are used, the technique is termed passive hemagglutination (PHA); with the use of latex particles, it is known as latex agglutination (LA).[15] In the Far East, an HIV gelatin particle agglutination test (PAT) is popular, offering good sensitivity, low cost, and ease of performance. It incorporates a quality control system to detect nonspecific antibodies directed toward the gelatin particles themselves, and results can be obtained with 2 hours with minimal hands-on time. Although appropriate for use in facilities with limited testing capabilities, this test must be performed under temperature-controlled conditions.[16]

HIV Confirmatory Test

Most testing algorithms require the use of very specific assays, such as the Western blot, indirect fluorescent antibody assay (IFA), or the radio-immuno-precipitation assay (RIPA), to verify the reactive screening test results. If performed and interpreted correctly, these extremely specific tests should not produce biologic false-positive results. However they are both laborious and expensive than screening assays. The primary purpose of confirmatory tests is to ensure that uninfected individuals who test reactive by screening assays are not incorrectly identified as being HIV infected.

Western Blot Test

The Western blot is probably the most widely accepted confirmatory assay for the detection of antibodies to the retroviruses; Many authorities consider it as the "gold standard" for validation of HIV results. It adopts the electrophoresis technique to separate HIV antigens derived from a lysate of virus grown in culture. This technique first denatures the viral components then imparts a negative charge to the antigens, and separates them according to their molecular weights. The separation of antigens in the technique allows for the identification of specific antibodies to each of the viral antigens in a subsequent set of steps similar to ELISA methodology.

Depending on particular antibodies in the sample, reactivity with the separated antigenic components results in band profiles. The type of profile (the combination and intensity of bands that are present) determines whether the individual is considered positive for antibodies to HIV. The classification of Western blot results is determined by certain criteria. Most institutions now follow the CDC guidelines that require reactivity to at least two of the following antigens: p24, gp41, gp120/160 for a positive classification. It is now universally accepted that a negative result is the absence of all bands. Two organizations, however, including WHO, suggest that results

can also be reported as negative if there is only a very weak p17 band. Indeterminate classifications occur when there is reactivity to one or more antigens, but not fulfilling the criteria for positivity.[1,2]

Unfortunately, sera from some non-infected individuals show some reactivity to one or more antigens if tested by Western blot. This reactivity may occur in as many as 15% of normal non-infected persons, and many times occurs in persons who are non-reactive by screening assays. Therefore, if ELISA non-reactive sera are tested by Western bolt, many will result in an indeterminate profile. Most indeterminate results show only weak reactions to the gag proteins (mostly p17, p24 and/or p55); other patterns occur but are less frequent. Any Western blot reactivity that does not meet the requirements for being positive or negative must be considered indeterminate. The significance of an indeterminate Western blot result varies depending on the risk factors, clinical status of the patient, and the Western blot profile produced.[3]

Indirect Immunofluorescent Antibody Assay (IFA)

In this technique, cells usually lymphocytes are infected with HIV and are fixed to a microscope slide. Serum containing HIV antibodies is added to react with the intracellular HIV. The slide is washed and then allowed to react with anti-immunoglobulin antibodies with a covalently bound fluorescence label attached. The reaction is visualized by using a fluorescent microscope. This technique has the advantage of providing definitive diagnosis of samples that have yielded indeterminate results by Western blot analysis. Disadvantages to its applications include the requirement of an expensive microscope and a subjective interpretation, thus necessitating well-trained individuals.

Alternative Confirmatory Strategies Using Screening Tests

Western blot or indirect fluorescent antibody technologies are widely used as in most industrialized countries, confirmation of HIV infection is accomplished using. In developing countries, these assays may be available in reference laboratories. It is common to find alternative confirmatory strategies for cost savings. Several investigators have verified that similar predictive values can be obtained by using two screening assays in tandem. This method can result in up to 80% cost savings.[1,8] It is important to select appropriate tests, with the most sensitive tests used in the initial testing. These strategies recommend initial screening using ELISA or a rapid/simple assay, followed by a second ELISA or repid/simple assay; the initial and second tests must be of different principle (bead versus micro-titer) and/or use a different antigen source.

HIV Testing in Hong Kong

Although, HIV/AIDS is not a modifiable disease in Hong Kong, a voluntary reporting system is adopted there. This is a case-based HIV/AIDS surveillance system, which comprises two parts: voluntary reporting by doctors and laboratory reporting. Data collected through the two systems are collected, matched and analyzed on a quarterly basis.

Medical doctors in Hong Kong are encouraged to supply information of newly diagnosed HIV infection and/or AIDS to the AIDS Unit of the Department of Health. Initially, a preliminary HIV/AIDS reporting form DH2293 was introduced in 1991 to systematically collect details such as the demographic characteristics, suspected exposure category, clinical status, and AIDS defining illness of the infected patients. After promotion and consolidation, reporting through DH2293 form was fully implemented and documented since 1995. Each reported case is assigned a unique code to facilitate subsequent follow-up of complications. Anonymity and confidentiality are strictly preserved in this system.[1,9]

The second source of data is obtained from voluntary private laboratory reporting since 1985. These laboratories that provide confirmatory HIV antibody tests , submit reports of the infected patients. Currently, three laboratories, Government sector, participate in the system. They are the Virus Unit of the Department of Health, Queen Elizabeth Hospital and Prince of Wales Hospital. Specimens screened HIV positive at private sector could be sent to the Department of Health virus laboratory for confirmation. There is only one private laboratory performing confirmatory HIV testing which does not participate in the laboratory surveillance system. In other words, this laboratory surveillance should provide a good coverage of the newly confirmed HIV positive cases in Hong Kong SAR. Laboratory reporting system enhances the accuracy of HIV surveillance through anonymous voluntary reporting by minimizing (1) double counting through case matching and (2) under-reporting in the private sector.[1,9]

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