California Association
for
Medical Laboratory Technology
Distance Learning Program

An Introduction to HIV, HIV Infection, and AIDS - 2014

Course # DL-968
by
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An Introduction to HIV, HIV Infection, and AIDS - 2014

Course Number DL-968
3.0 CE
Level of Difficulty: Basic

Elizabeth Crabb Breen, M.T. (A.S.C.P.), Ph.D.
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MEASURABLE OBJECTIVES:
Upon completion of this course, the reader should be able to:

- identify the cells in the body that can be infected by Human Immunodeficiency Virus (HIV)
- list the ways in which HIV is transmitted
- identify the precautions necessary to reduce occupational exposure and prevent infection
- describe the screening and confirmatory tests used for diagnosis of HIV infection

Author’s Note: Dr. Janis V. Giorgi, an HIV/AIDS researcher at UCLA in the early days of the epidemic, passed away at an early age from cancer. This course is dedicated to her memory in honor of her contributions and of the many men and women she inspired to work in the field of HIV/AIDS research.

FREQUENTLY USED ABBREVIATIONS
AIDS Acquired Immunodeficiency Syndrome
HIV Human Immunodeficiency Virus
WBC White blood cells
CD4 Cell surface molecule found on certain WBC; primary cellular receptor for HIV
T_H T helper cell (CD4-positive T cell)
IL-2 Interleukin-2
CTL-P Cytotoxic T lymphocyte (CTL) precursor (CD8-positive T cell)
CTL Cytotoxic T lymphocyte
PCP Pneumocystis pneumonia
KS Kaposi's sarcoma
HAART Highly-active anti-retroviral therapy introduced in 1995; now also known as combination anti-retroviral therapy (cART)
CDC The Centers for Disease Control and Prevention
PHS Public Health Service (United States)
HCP Health care personnel
PCR Polymerase chain reaction
PEP Post-exposure prophylaxis
PrEP Pre-exposure prophylaxis
IA Immunoassay
NAT Nucleic acid testing
INTRODUCTION

Since its recognition as an emerging clinical syndrome in the summer of 1981 (1), Acquired Immunodeficiency Syndrome (AIDS) has impacted the delivery of medical care at all levels. As the transmission pattern of AIDS emerged, followed by the isolation of its etiologic agent, Human Immunodeficiency Virus (HIV), assessment of HIV infection and prevention of occupational exposure became issues of great importance to clinical laboratory scientists. Over the course of more than three decades since HIV and AIDS became part of the medical landscape, an extraordinary amount has been learned about the virus and its effects on the body. This is due, at least in part, to technical advances that have improved our understanding of and response to HIV infection and disease progression. The purpose of this course is to introduce and/or review several basic aspects of HIV infection and disease in order to assist clinical laboratory scientists in understanding and dealing with issues relevant to providing care for persons living with HIV.

In order to lay a foundation for understanding how and why HIV affects the immune system, the course will first briefly review how the immune system responds under normal conditions. Then an historical perspective on the HIV/AIDS epidemic will provide an overview on the subject. It will be followed by a discussion of the basic elements of HIV virology and transmission, with a special emphasis on occupational exposure for clinical laboratory workers. The final section of the course will describe the continuum of HIV infection and some of the laboratory tests used to diagnose and follow HIV-infected individuals along the continuum.

NORMAL IMMUNE RESPONSES

The immune system is very complex, consisting of an array of cells, tissues, and organs that collectively protect us from the bewildering assortment of pathogens and other foreign materials to which we are constantly exposed. This remarkable system is the subject of another CAMLT distance learning course, where the cellular interactions necessary for normal immune responses were reviewed in some detail (2). In order to understand how and why HIV interferes with the proper functioning of the immune system, it will be helpful to briefly review what happens in a normal immune response.

The white blood cells (WBC) circulate in the blood and lymph fluid and migrate through the tissues. They are the key to the ability of the immune system to respond to foreign materials (known as antigens) that manage to get past the skin and/or other physical barriers, and enter the body. Some WBC such as neutrophils, monocytes, and macrophages, are non-specific scavenger cells that attempt to remove any debris or organisms by engulfing or phagocytosing, then digesting and eventually disposing of the unwanted material. They provide a rapid (but generic) first line of defense inside the body against antigens of all types. However, it is the antigen-specific lymphocytes upon which we depend for highly-focused and reproducible responses against specific antigens, including infectious agents (1).

There are multiple subsets of lymphocytes that are involved in antigen-specific responses, i.e., immune responses that selectively recognize, eliminate, and remember individual antigens. T and B lymphocytes (also known as T and B cells) are capable of binding and responding to individual antigens in a highly specific manner, and, ultimately, give rise to two different types of immune responses. B cells bind soluble (extracellular) antigens in the circulation and secrete antibody in response (humoral immunity), while T cells recognize cell-associated antigens and
either provide help to other cells (T helper or T<sub>H</sub>) or generate cytotoxic T lymphocytes (CTL) capable of killing infected or defective cells (cell-mediated immunity).

Figure 1: The Generation of Humoral and Cell-Mediated Immune Responses. A schematic illustration of the cellular interactions necessary to generate antigen-specific immune responses. The antigen in question is a virus (*), which is shown both as free-floating viral particles and in infected cells. The hatched cell is a virally-infected cell that has been killed by a CTL. Abbreviations are as follows: interleukin-2 (IL-2), immunoglobulin (Ig), antigen presenting cell (APC), T helper cell (T<sub>H</sub>), B cell (B), CD8-positive T cell/CTL precursor (CTL-P), cytotoxic T
A single lymphocyte does not act in isolation when responding to antigen. Rather, any one antigen-specific lymphocyte is dependent on interactions with other cells in order to successfully mount a specific response (Figure 1). Regardless of whether a B cell or a CTL precursor (CTL-P) first encounters antigen, neither of these lymphocytes can respond fully to that antigen without the presence and assistance of a T<sub>H</sub> cell.

T<sub>H</sub> cells, while similar in appearance to CTL-P and B cells, can be distinguished from the other lymphocytes by the presence of a cell surface molecule known as CD4 (1). The CD4 molecule not only serves as a marker of the helper cell population, but is physically involved in the recognition of antigen by T<sub>H</sub> cells. (In parallel, there is a different cell surface molecule on CTL-P cells, CD8, that distinguish these cells as precursors to cytotoxic or killer T cells and participates in the recognition of antigen.) Following successful recognition of antigen on the surface of an antigen-presenting cell (APC), a single T<sub>H</sub> cell will respond by secreting a cytokine known as interleukin-2 or IL-2. Cytokines, in general, are protein messenger molecules which send signals between cells. IL-2 signals the T<sub>H</sub> cell which produced it to undergo proliferation, or cell division, which gives rise to a number of progeny T<sub>H</sub> cells, all of which are specific for the same antigen as the original parent cell. In addition, IL-2 can diffuse to nearby B and/or or CTL-P cells that have recognized antigen, and deliver critical signals necessary for those cells to respond to the presence of antigen. Therefore, the presence of T<sub>H</sub> cells with the ability to secrete IL-2 in response to antigen is essential to initiate both humoral (B cell) and cell-mediated (CTL) responses.

Following the initial secretion of IL-2 by T<sub>H</sub> cells in response to antigen, T<sub>H</sub> cells secrete additional cytokines, which provide further help to B and/or CTL-P cells that have bound antigen. This allows B cells to fully develop into antibody-secreting plasma cells, and CTL-P cells to become fully-functional cytotoxic T lymphocytes (CTL) capable of killing other cells. This process of maturing and acquiring new function is known as differentiation. In the case of B cells, T<sub>H</sub> cells provide help by establishing antigen-specific cell-to-cell contact, delivering both cell-surface signals and cytokines. In the case of CTL-P cells, no physical contact is necessary. Rather, the T<sub>H</sub> provides help solely through the secretion of IL-2 and other cytokines in close proximity to the CTL-P cell that has also recognized antigen on the surface of the same APC. As a result of one or the other or both of these types of interactions, humoral and/or cellular immunity is/are generated in response to an antigen, which hopefully eliminates (or at least controls) the antigen and/or pathogen. In addition to dealing with the antigen at the time of first exposure, antigen-specific immune responses by T<sub>H</sub>, CTL, and B cells also give rise to memory B and T cells, which allows the immune system to mount a faster, much more effective response on subsequent exposures to the same antigen(s). It is this successful “immunologic memory” which provides immunity, i.e., the protection from the same infectious disease after initial exposure and recovery (1, 2).

AN HISTORICAL PERSPECTIVE ON AIDS and HIV

The discipline of virology (the study of viruses) deals with very small infectious agents that are dependent on host cells to survive and reproduce. Due to their extremely small size, viruses cannot be visualized using a light microscope. Therefore, virologists must utilize electron microscopy to find and examine viral particles. Since viruses reproduce only when inside an appropriate host cell, they are often notoriously difficult to isolate and culture in the laboratory. Not surprisingly, many advances in virology have come about only when new or improved
technology became available. This was very much the case in December of 1980, when new culture techniques enabled the successful isolation of Human T cell Leukemia Virus-1 (HTLV-1) (Table I). This was a significant advance because HTLV-1 was the first human retrovirus ever isolated. Retroviruses are a class of viruses with a unique life cycle (as will be explained in a later section) that posed particular challenges to virologists attempting to culture them. The isolation of a human retrovirus was a notable accomplishment in itself. In retrospect, however, the success with HTLV-I turned out to be extremely important and timely, as it laid the foundation for attempts to identify the cause of a mysterious immunodeficiency that was first recognized only months later.

In June of 1981, a cluster of cases of what was then known as Pneumocystis carinii pneumonia (PCP), a rare infection usually seen only in immunocompromised individuals, was reported in the Los Angeles area in relatively young homosexual men with no previous history of immunodeficiency (1, 3). This report was followed immediately by others in the New York and San Francisco areas, where similar clusters of PCP had been seen, along with cases of Kaposi’s sarcoma (KS), a rare cancer usually seen only in men over sixty years of age. These cases of PCP and KS were the first of what came to be known as Acquired Immunodeficiency Syndrome or AIDS. It was soon recognized that this syndrome, characterized by PCP, KS, and other unusual infections typically seen in association with immunodeficient states, was spreading in much the same pattern as had been observed for Hepatitis B—via homosexual and heterosexual contacts, through receipt of blood transfusions or blood products (such as Factor VIII concentrate for hemophilia), and from mother to infant. The similarity to Hepatitis B strongly suggested that a viral agent was involved, leading to an intense search for an AIDS-related virus. As an historical footnote, it should be mentioned that the organism responsible for PCP was originally thought to be a protozoan capable of infecting multiple mammalian species, but is now widely recognized to be a fungus that infects only humans. As a result, a new name for the organism has been introduced, Pneumocystis jiroveci, but the term PCP has been retained to mean “Pneumocystis pneumonia” (see http://www.cdc.gov/ncidod/EID/vol8no9/02-0096.htm).

The first isolate of a putative “AIDS virus” was recovered not from a patient with AIDS, but with persistent lymphadenopathy, a swelling of the lymph nodes that was considered a precursor to AIDS (part of the so-called “AIDS-related complex” or ARC, a term which is now considered obsolete, but was then used to describe a number of symptoms that preceded AIDS). Named “lymphadenopathy-associated virus” or LAV, the virus was isolated in late 1983 by a team led by Dr. Luc Montagnier at the Pasteur Institute in France (Table I). In early 1984, a team led by Dr. Robert Gallo at the National Institutes of Health in the U.S. isolated a virus from a patient with AIDS that was identical in appearance to LAV. Since it was Dr. Gallo’s laboratory that had isolated HTLV-1 in 1980, and the new virus appeared to be a similar retrovirus, the AIDS-associated virus isolated in Gallo’s laboratory was named HTLV-III (HTLV-II had been isolated in the interim). At nearly the same time, a team led by Dr. Jay Levy at University of California, San Francisco, also isolated a virus from an AIDS patient, known as “AIDS-related virus” or ARV. In an effort to standardize the nomenclature regarding the AIDS-associated viruses, a consensus name, “human immunodeficiency virus” or HIV, was agreed upon in 1986. The names of the original isolates, as well as hundreds of other isolates of HIV, are still used to identify the individual and unique strains of HIV. A related human retrovirus that causes a less severe immunodeficiency was isolated and identified in 1986, leading to the designation of the
original virus as HIV-1, and the more recently described virus as HIV-2. For the remainder of this course, HIV-1 will be referred to simply as HIV, unless otherwise noted.

Once the putative etiologic agent of AIDS had been isolated, the most pressing need was to develop a means of screening for HIV infection to protect the blood supply used for transfusions and blood products. It took slightly more than a year to develop and implement an HIV-antibody test, using enzyme-linked immunosorbent assay (ELISA) technology, for use in blood banks. HIV-1 antibody screening in the United States began in March of 1985. HIV testing will be discussed in more detail in a following section.

Following the isolation of HIV in 1983-84 research efforts focused on characterizing the structure and life cycle of the virus, and on trying to understand how changes in the immune system are brought about by infection with HIV. After the initial burst of new information regarding HIV, especially regarding its structure and retroviral life cycle, progress in treating and evaluating this viral disease has been like many others—painfully slow at times, and dependent on technical advances for clinical and/or scientific breakthroughs.

With an understanding of the HIV retroviral life cycle, the mid 1980s saw the clinical trial and approval of the first anti-retroviral drug for the treatment of AIDS, AZT (also known as zidovudine or Retrovir™). While other similar drugs would be approved over the next few years for use in place of AZT (ddI [Videx™], 3TC [Epivir™]) or in combination with AZT (AZT+3TC or AZT+ddI), it would be the mid-1990s before any antiviral treatment was offered to HIV-infected pregnant women, and before any new types of highly effective antiretroviral drugs became available to all HIV-infected persons. In the interim, there were technical advances in testing with new generations of HIV-1 antibody ELISA tests, and the introduction into blood banks of a combination HIV-1/HIV-2 ELISA and an HIV antigen test.

Pediatric HIV infection due to blood transfusion was virtually eliminated in the U.S. in 1985 with the introduction of antibody screening of donated units, leaving the transmission of HIV from mother to infant during pregnancy and/or birth as the source of pediatric AIDS cases. In 1994, the first major breakthrough in the prevention of pediatric HIV infection came as treatment of HIV-infected pregnant women with AZT was shown to be both safe and effective in reducing transmission of HIV during pregnancy and delivery.

1995 marked the beginning of widespread use of an entirely new class of antiretroviral drugs known as protease inhibitors (1). The rapid evaluation of these powerful new drugs was possible only because of a technical development that paralleled the pharmacological advances. This was the development of quantitative determinations of HIV viral nucleic acid in serum or plasma specimens, specifically HIV RNA (the genetic material of the virus). As described in more detail later in this course, these measurements permit the calculation of the concentration of virus present in an individual’s blood, rather than just detecting the presence of antibody to the virus. In the most commonly used nucleic acid test (NAT), the quantitation is performed by polymerase-chain reaction (PCR), and is expressed as viral copies per milliliter of plasma. This kind of measurement, known as plasma HIV RNA viral load, or more simply as HIV viral load, could quickly indicate if an antiviral drug was succeeding or failing in controlling the level of HIV found in the blood. This was a tremendous advance over having to wait months or years for clinical endpoints (such as an AIDS diagnosis or death) indicating treatment success or failure. Viral load testing demonstrated that the new protease inhibitor drugs were most likely to succeed when given in combination with AZT and/or other existing drugs, and so helped to usher in the era of the “anti-HIV cocktail” or “highly-active anti-retroviral therapy” (HAART). More
recently, treatment of HIV infection with a combination of anti-viral drugs is sometimes described as “combination anti-retroviral therapy” or cART.

As noted in the AIDS and HIV Chronology (Table I), there was a dramatic decline (63%) in the U.S. in deaths from HIV/AIDS between 1995 and 1998, following the introduction of HAART/cART (4). Similarly, new AIDS cases in the U.S. declined by 45% from 1993 to 1998. This led to a mistaken (and very premature) assumption among many persons in this country that, because of new antiviral therapies, the rate of AIDS cases and deaths would continue to decline, and therefore, HIV infection and/or AIDS no longer presented a threat to public health. In reality, according to the statistics reported by the Centers for Disease Control and Prevention (CDC), the federal agency responsible for tracking HIV infection and AIDS (among a host of other infectious diseases), the estimated numbers of new HIV infections in the U.S. has remained relatively stable at about 50,000 infection per year since the mid-1990s (3).

Introduction of additional anti-viral drugs, improvements in HIV viral load testing for clinical purposes and NATs for blood bank use, and expanded access to HIV treatments around the world contributed to incremental progress in monitoring and controlling HIV infection and disease during the early 2000s. By 2010, scientific studies confirmed one of the greatest hopes for combination anti-retroviral therapies: HIV treatment (and subsequent reduction in HIV viral load) in an infected person dramatically reduced the likelihood that person would transmit HIV to an uninfected sexual partner (1, 4). This revolutionized the view of the role of HIV treatment worldwide (“treatment as prevention”), resulting in increased resources and even greater access to HIV testing and treatment in many countries, and trends toward fewer new HIV infections and fewer AIDS-related deaths (www.unaids.org). From 2008-2011 (the latest years for which CDC estimates are currently available), the estimated annual number of new AIDS cases in the U.S. has remained stable, while the number of deaths in persons with an AIDS diagnosis has continued to decrease (5). Additional details on the topics of anti-HIV therapy and statistical trends of the HIV/AIDS epidemic are beyond the scope of this introductory course, but are presented in a second course on HIV/AIDS (6). It is clear, however, that after three decades of the HIV/AIDS epidemic, its history is still unfolding, with HIV infection, treatment, and disease continuing to place a significant burden on healthcare systems.

**HIV VIROLOGY**

All viruses, including HIV, cause symptoms and/or disease because they act as parasites, infecting and then affecting particular cells of a host organism. The symptoms and diseases associated with a particular virus are dictated by the type of cell which can be infected by that virus. In turn, the ability of a virus to bind to and gain entry into a particular cell type is a function of the structure of the virus. Therefore, in order to understand how HIV gains entry into its host cells, we need to examine its structure.

Like all viruses, HIV is a very simple organism, consisting of genetic material (two identical strands of RNA) contained within a protein shell or coat, which is also known as the viral envelope (Figure 2) (1). Protruding through the viral coat are proteins which are glycosylated (i.e., have sugars attached), and so are called glycoproteins (gp). Viral proteins are identified by their size, so the two glycoproteins on the surface of HIV, which have molecular weights of 41 and 120 kilodaltons (Kd), are known as “gp41” and “gp120”. Within its viral core, HIV carries three viral enzymes that are essential for its lifecycle: reverse transcriptase, integrase, and protease.
HIV is capable of binding to and infecting any human cell that has the CD4 molecule on its cell surface (1). This is accomplished by a direct interaction between a gp120 molecule on the exterior of an HIV viral particle, and the CD4 molecule on the surface of a cell. CD4, therefore, acts as the primary receptor molecule that allows HIV to bind to the surface of a potential host cell. This means that the critical T\textsubscript{H} cell population, which is necessary for all antigen-specific immune responses, is a major target for infection by HIV. In addition, CD4 is expressed at low levels on monocytes and macrophages, a type of antigen-presenting cell known as a dendritic cell, and macrophage-like cells in the brain known as glial cells, allowing all of these cell types to also serve as hosts (and, perhaps more importantly, as long-lived reservoirs) for HIV.

The viral lifecycle of HIV is discussed in detail in another course (6), but it is necessary to briefly review it here to provide a basic understanding of the biology of the virus. Once HIV has attached to a cell via CD4, additional co-receptor molecules on the surface of potential host cells interact with CD4 and HIV to permit the virus to enter the cell (1). Inside the cell, HIV initiates a unique viral life cycle that is found only in viruses that are members of the retrovirus family. As the name implies, retroviruses are virus that are “retro” or work in reverse. This distinction refers to the method by which these viruses make copies of their genetic material (Figure 3). In all cells, the genetic material is DNA, which undergoes transcription into...
RNA, which is then translated into protein. Regardless of the cell type, this process goes only in this direction: DNA to RNA to protein. In retroviruses, the genetic material is RNA, and the first step of transcription goes in reverse of the normal cellular process, making DNA copies from viral RNA. This process, known as reverse transcription, requires an enzyme, reverse transcriptase, which is only made by retroviruses. As shown in Figure 2, HIV carries two molecules of reverse transcriptase with it into the cell, so that it is ready to make a DNA copy of the viral RNA.

The DNA copy of HIV, called a provirus, is transported to the nucleus of the host cell, where it uses the viral enzyme integrase to randomly integrate itself into the DNA that makes up the chromosomes of the cell. The proviral DNA will remain inactive or latent among the genes of the infected cell until the cell begins to respond to antigen and/or cytokines, which results in the production of new virus particles. These new viruses bud from the surface of the infected cell and circulate through the body, spreading the infection to other CD4-expressing cells. It is the amount of HIV RNA present in the viral particles circulating in the blood that is measured in determinations of HIV viral load.

**TRANSMISSION OF HIV**

Fortunately, HIV is a very fragile virus that dies rapidly outside the body. It is extremely susceptible to drying, and is easily killed by disinfectants (such as 10% bleach solutions, betadine, or rubbing alcohol). As a result, HIV is not transmitted by casual contact, but rather, only by one of three modes of transmission: blood (or infectious body fluid)-to-blood contact, sexual contact, and mother-to-infant during or immediately following pregnancy (3).

HIV is present in the blood of an infected individual both as free-floating viral particles and within infected CD4-positive T cells and monocytes/macrophages. Transmission of HIV to an uninfected individual is possible due to free viral particles as well as via transfer of HIV-infected WBCs (1). A single HIV particle, with a diameter of approximately 100 nanometers (or 0.1 micron), may be able to squeeze between intact epithelial cells, or would require only a submicroscopic hole or microabrasion in a protective barrier in order to pass through. An infected lymphocyte with a diameter of 10 microns would require an opening one hundred times larger than a viral particle. However, even an opening of this size would still be far below the size detectable by the naked eye. Therefore, *any* contact with blood from another person carries a risk of HIV infection, whether through the skin due to needle sharing or needlestick, or due to exposure of unprotected skin or a mucous membrane that appears perfectly intact. This includes not only the obvious shared needle use found among injecting drug users or accidental needle injury among health care personnel (HCP), but any type of shared needle use (tattooing, ear piercing, home vitamin injections), as well as any other kind of occupational or accidental exposure to blood or other body fluids containing significant amounts of virus and/or blood. Once through the skin or membranes, viral particles and HIV-infected WBC can encounter susceptible cells and/or gain access to the lymphatic vessels leading to lymph nodes, where a new infection can be established.

An obvious question that arises in light of the transmission of HIV via blood is the safety of blood transfusions and blood products. As shown in Table I, the blood supply in the U.S. has been tested for the presence of HIV-1 antibodies since 1985; a combination test for HIV-1 and HIV-2 antibodies was introduced in 1992 (3, 7). Due to the time needed for the immune system to generate detectable levels of HIV-specific antibodies following a new HIV infection, it was
estimated that antibody testing left open a window of time of approximately 22 days during which a blood donor could be HIV-infected, but not yet detectable by blood screening tests. Therefore, in 1996, a test for the HIV core antigen p24 (Figure 2) was added to U.S. blood bank screening. Although p24 is undetectable most of the time in an HIV-infected person, the p24 antigen test was a valuable addition to blood bank screening at that time because p24 is often detectable during the first few weeks of acute HIV infection, before the development of antibodies to HIV. It was estimated that the addition of the p24 antigen test reduced the window period to 16 days. Just as blood levels of p24 HIV antigen typically spike during acute HIV infection, HIV viral loads are known to peak in the first weeks following infection, prior to the development of HIV-specific antibodies (1). Beginning in 1999, a new nucleic acid testing (NAT) method capable of directly detecting HIV viral RNA (similar to PCR methods employed for HIV viral load testing), were introduced by the Red Cross as an investigational protocol in every donor unit collected nationwide. The FDA licensed the use of a duplex NAT screening assay that simultaneously detects both HIV-1 and hepatitis C virus in 2002, which was updated to include detection of hepatitis B virus in 2009 (7). This NAT technology (known as transcription-mediated amplification), plus HIV antibody screening, is now in use for every non-autologous blood donation in the U.S. According to the Red Cross, the addition of the HIV NAT reduces the window period by about 2 weeks compared to HIV antibody testing alone. This leaves a time period of approximately 7-10 days during which an HIV-infected donor may not be detected by current blood screening tests. HIV NAT is performed on pooled samples from 16 donors at a time, thus allowing a more efficient means of screening for such a low-probability positive sample. This testing scheme, combined with other improvements to minimize human and/or recordkeeping errors, has resulted in an estimated risk of HIV per transfused unit as 1 in 1.0-1.5 million (7). This is a four- to six-fold reduction in risk relative to the late 1980s, when the risk of HIV infection per transfusion was estimated to be 1 in 250,000. As a point of reference, the current risks of Hepatitis B and Hepatitis C infection per transfused unit are similar, estimated to be 1 in 800,000-1 million and 1 in 1 million, respectively. In this context, it is important to emphasize that, contrary to a common misconception, there is no risk associated with donating blood for transfusion purposes, as all equipment utilized is sterile and disposed of properly after a single use.

Clinical laboratory scientists are clearly at risk for occupational exposure to HIV, primarily through exposure to blood and visibly bloody body fluids (8). In addition, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid are considered potentially infectious, although it has not been possible to estimate the risk for HIV transmission by these fluids. It is appropriate, therefore, in the context of a discussion on HIV transmission, to review what is known about occupational transmission of HIV among HCP, and what steps can be taken to reduce risk of exposure to and infection by HIV.

If and when occupational exposure does occur with blood from a person known to be HIV-infected, it usually does not result in infection. The estimated risk of infection due to known HIV-infected blood is estimated to be 1 in 200 to 1 in 500 for percutaneous exposures such as needlesticks, approximately 1 in 1000 for mucous membrane exposures, and less than 1 in 1000 for exposures to non-intact skin (chapped, abraded, or affected by dermatitis) (8). The risks of HIV infection by occupational exposure to fluids/tissues other than blood are probably lower than those estimated for blood. In the more typical case, where the HIV infection status of the patient involved is not known, one would expect that the risk of infection from a single
blood/fluid exposure would be much lower, depending on the prevalence of HIV infection in the patient population served. It is important to note that occupational HIV infection has been documented to have occurred following a single needlestick exposure. However, a lack of infection has also been documented following multiple needlesticks in a single individual.

According to the CDC, there have been rare cases of occupationally acquired HIV infection of HCP (9). In this context, healthcare personnel are defined by the U.S. Public Health Service (PHS) as “…all paid and unpaid persons working in healthcare settings who have the potential for exposure to infectious materials, including body substances (eg, blood, tissue, and specific body fluids), contaminated medical supplies and equipment, and contaminated environmental surfaces.” (8) In addition, occupational exposure (and appropriate clinical follow-up) would include workers who have had any direct contact without barrier protection to concentrated HIV in a research laboratory or production facility. Among HCP for whom investigations were completed from 1981-2010, there have been only 57 documented occupationally acquired HIV infections, and another 143 possible occupationally acquired infections (9). Documented infections are those in which: 1) an HIV seroconversion event occurred within an expected time frame following an exposure to an HIV-infected (HIV-positive, HIV+) source, and, 2) the exposed HCP had no known non-occupational risk factors for HIV infection. Possible occupationally-acquired infections are those in HCP found to be HIV+, with opportunities for occupational exposure and no known non-occupational risk for HIV. The CDC has not reported any new documented occupationally acquired cases among HCP since 1999; the most recent possible case occurred in 2009. While any such infections are regrettable, keep in mind that 57 occupationally acquired HIV infections represent a very tiny proportion of the tens of thousands of health care professionals that are potentially exposed across the country, yet remain HIV-uninfected (HIV-). At the same time, there are many HCP who are HIV+, but more than 90% reported non-occupational risk factors for acquiring their infection.

Among the 57 documented occupationally acquired HIV infections, it is not surprising that the vast majority of infections were attributed to exposure to HIV-infected blood (49/57); the remaining exposures involved other fluids or concentrated laboratory virus preparations. Percutaneous exposure (puncture through the skin, as in a needlestick injury) was the most common route of infection (48/57), and the most infections occurred among nurses (24/57), followed by clinical laboratory personnel (16/57). For more details on occupationally acquired HIV infections among HCP, see reference 9.

As is true for all blood borne pathogens that may be encountered by a clinical laboratory scientist, the best protection against occupational exposure to HIV is Standard Precautions, i.e., the handling of anything containing blood and/or body fluids as potentially infectious, regardless of the source patient (8). In the 1980s, AIDS was first entering the consciousness of clinical lab professionals, but hepatitis was already well-established as a health threat. Some clinical laboratory scientists may recall how, at that time in the clinical laboratory and throughout the hospital, the proper handling and disposal of used needles and syringes, and routine wearing of latex gloves might have been described as occasional rather than universal! However, with increasing awareness of HIV, as well as Hepatitis B and C (formerly known as non-A, non-B hepatitis), many phlebotomy and laboratory procedures, equipment, and protocols have been modified over the years to emphasize increased safety and reduced occupational transmission of blood borne agents. In addition to the changes associated with universal blood precautions, such as mandatory use of gloves and other personal protective equipment, and proper handling and
disposal of samples, needles, and syringes (known as work practice controls), occupational transmission can be reduced by well-designed and well-maintained work areas that reduce clutter and minimize the chance of accidental spills and/or exposures.

At various times, especially in the earlier days of the HIV/AIDS epidemic, arguments were made for testing all patient samples for the presence of HIV antibodies as a means of protecting HCP. However, truly universal laboratory precautions have been and will continue to be the best protection against occupational exposure to HIV for clinical laboratory scientists, *not* universal testing of patients. As mentioned above regarding testing of blood donated for transfusion purposes, there is a window period early in HIV infection during which HIV antibody testing or even the combination of antibody and nucleic acid testing would not detect an HIV-infected person. Any mass screening of patients would likely miss such HIV-infected persons, leading to a false sense of security that all “negative” patients were not infectious and a potentially dangerous relaxation of infection precautions.

In its 2013 update to its guidelines for the management of HCP exposures to HIV, the US Public Health Service (in conjunction with the CDC) stated “Preventing exposures to blood and body fluids (i.e., primary prevention) is the most important strategy for preventing occupationally acquired HIV infection…For instances in which an occupational exposure has occurred, appropriate postexposure management is an important element of workplace safety.” (8) All institutions that deal with human blood and/or body fluid samples should have an established and well-publicized protocol that is to be followed in the case of a HCP exposure to known or suspected HIV-infected blood and/or fluids. It is important to emphasize that HCP need to be aware of and informed about such a protocol before an accidental exposure, because the most thorough protocol in the world is of no use to someone who is unaware of its existence. If there is resistance to establishing and/or educating employees about such protocols, administrators should consider the ramifications of dealing with and/or caring for an employee infected with HIV on the job in the absence of such a protocol. The PHS recommendations state that “both individual healthcare providers and the institutions that employ them should work to ensure adherence to Standard Precautions…” and, in the event of an occupational exposure, to ensure prompt and appropriate management (i.e. treatment and follow-up of the exposed HCP) and reporting. Postexposure protocols should include, at the very minimum, instructions to wash potentially-exposed wounds and skin sites with soap and water; mucous membranes should be flushed with water.

In a major change from its 2005 guidelines, the PHS no longer recommends that the severity of exposure be used to determine if post-exposure prophylaxis (PEP) is to be offered to a potentially HIV-exposed HCP (8). PEP refers to combination anti-retroviral drug therapy for the purpose of preventing HIV infection following a possible exposure. According to the updated recommendations, PEP is now recommended routinely for *all* occupational exposures to HIV, and should consist of a combination of three (or more) drugs from a specified list (see Appendix A, reference 8). PEP is not necessarily the same combination and/or dose of drugs that would be used to treat an established infection (as in HAART or cART), but rather, uses drugs chosen specifically to prevent infection following exposure. In order to be most effective at preventing HIV infection, PEP should be started *as soon as possible* after the exposure (ideally, within the first hours), and should be continued uninterrupted for 4 weeks. Therefore, it is essential that a procedure for seeking and receiving PEP be clearly established, so that it can be administered in a timely fashion. The change in the recommendations reflects the advances in the anti-retroviral
drugs now available for PEP, which are better tolerated with fewer side-effects. Therefore, there is less risk in providing PEP to all exposed HCPs, and perhaps more importantly, makes it more likely that an exposed HCP who has started PEP will complete the recommended 4 week course. 

Postexposure protocols should include investigating the HIV status of the exposure source patient, in order to guide the appropriate use of HIV PEP for the exposed HCP. If the source’s HIV status is unknown, this could include rapid HIV testing, conventional ELISAs, or third generation chemiluminescent assays for HIV antibody, or fourth generation combination HIV ab and p24 antigen assays, depending on the resources available. Regardless of which type of HIV testing is employed or the timeliness of results, administration of PEP should not be delayed while waiting for test results (8). It is important to emphasize that if no information regarding HIV status is available in the medical record of the source person at the time of exposure and/or is not willingly provided by the source person, all applicable laws regarding HIV testing and confidentiality must be respected. If the source patient is determined to be HIV negative, PEP should be discontinued, and no follow-up HIV testing for the exposed HCP is indicated.

In light of the complexity in choosing and administering HIV PEP, the precise regimen for each exposed HCP should be determined whenever possible in consultation with either an infectious disease specialist or another clinician with expertise in the use of antiretroviral agents. Once again, the PHS recommendations emphasize that such a consultation should not delay initiating PEP. Regardless of whether an exposed HCP is taking PEP, he or she should be followed closely, with the first re-evaluation within 72 hours of the HIV exposure. All exposed HCP should be advised to use precautions to prevent secondary transmission, especially during the first 6-12 weeks after exposure. HCP who have initiated PEP should be advised of the importance of completing the recommended 4-week regimen, and be provided with appropriate information regarding potential side effects, possible drug interactions, etc. Psychological counseling should be an essential component of the follow-up, as the psychological impact of needle injury or other exposure to blood or body fluid should not be underestimated.

All postexposure protocols should provide a mechanism for obtaining a baseline blood sample from the exposed HCP for HIV testing, complete blood counts (CBC), and renal and hepatic function (8). Documenting the HIV status of the exposed HCP at the time of the exposure is for the protection of both the HCP and the institution, and should respect all applicable laws regarding informed consent by the HCP and confidentiality of HIV antibody testing. In addition, pregnancy testing should be offered to all women whose pregnancy status is unknown. Exposed HCP should receive follow-up counseling, postexposure testing, and medical evaluation regardless of whether they receive PEP. CBC, renal, and hepatic function tests should be repeated 2 weeks postexposure, and then as indicated if abnormalities are seen. The PHS recommends that HIV-antibody testing be repeated at 6 weeks, 12 weeks, and 6 months following the exposure. If the testing is performed utilizing one of the more sensitive fourth generation combination HIV antibody/p24 antigen tests, HIV monitoring can be concluded at 4 months postexposure. Testing is intended to check for evidence of seroconversion, i.e., a change in serostatus from HIV-antibody negative (HIV-) to HIV-antibody positive (HIV+), indicating recent HIV infection. The PHS recommendations emphasize that all HIV testing results should be given to the exposed HCP at face-to-face appointments. For additional details on the PHS recommendations for management of occupational exposures and PEP, please see reference 8.
While blood accounts for a significant proportion of HIV transmission, especially in occupational exposures, it is definitely not the only body fluid that is capable of HIV transmission (1, 3). Similar to blood, semen and vaginal secretions from HIV-infected individuals can contain sufficient levels of both free virus particles and HIV-infected WBC to transmit the virus from one person to another. This occurs, of course, in the context of sexual contact, which is estimated to be responsible for 75% of all HIV transmission worldwide (1). By its very nature, sexual activity involves delicate mucous membranes that are subjected to physical stresses that can easily cause microscopic breaks and tears in the membranes. Therefore, any kind of sexual activity that results in exposure to semen or vaginal fluid, regardless of the anatomic location of such an activity, carries a risk of HIV infection. Once HIV particles or HIV-infected WBCs carried in semen or vaginal fluid pass through skin or membranes, the situation is the same as with bloodborne transmission--they can encounter susceptible cells and/or gain access to the lymphatic vessels leading to lymph nodes, where a new infection can be established. For purely anatomical reasons, i.e., that semen remains in the vagina for extended periods of time after vaginal intercourse, it is twice as likely that a woman will become infected by an HIV+ male partner compared to a man being infected by an HIV+ female partner (1). Similarly, receptive partners in anal intercourse have a greatly increased risk of HIV infection. It has also been shown that men who are circumcised are 60% less likely to become infected with HIV by sexual contact, possibly because removal of the foreskin prevents the retention of susceptible cells; circumcised and uncircumcised HIV+ men are equally likely to pass HIV to an uninfected partner (1, 4).

It is not surprising that the presence of other sexually-transmitted diseases (STDs), especially those that cause genital lesions such as herpes, gonorrhea, or syphilis, greatly increase both the risk of transmission of and infection by HIV as a result of a single sexual encounter. Persons who have an STD are two to five times more likely to become infected when exposed to an HIV+ sexual partner, while an individual with both HIV and STD is more likely to transmit HIV than someone who is HIV+ but does not have another STD (3). STDs increase the number of WBC present in the semen and vaginal fluid, increasing both the possible source of and possible target for HIV infection, and open lesions provide easy access to susceptible cells and the lymphatic system. However, important inroads have been made in reducing the risk of sexually-transmitted HIV. It is now well-established that treatment of an individual’s HIV infection with cART can drastically reduce the likelihood of sexual transmission of HIV, presumably by reducing the amount of HIV present in semen and/or vaginal fluid. In the first study to document this observation, in more than 1700 heterosexual couples with one HIV+ and one HIV- partner, sexual transmission of HIV was reduced by 96% if the infected partner was receiving cART (4). In many situations, uninfected women are exposed to HIV+ sexual partners who are not on treatment, and who may refuse to use protective measures such as latex condoms. Therefore, it was an important advance for women to be able to protect themselves when the use of a vaginal gel containing an anti-HIV drug (tenofovir) was shown to reduce acquisition of HIV from an infected partner via sexual contact by 39% (4). More recently, very different types of studies have shown that sexual transmission of HIV between men who have sex with men can be reduced by 60-70% if both the infected and the uninfected partner regularly and consistently take a specific combination of anti-viral drugs as a preventative measure (1). This approach, which is controversial due to cost, potential side effects of the drugs in otherwise healthy uninfected individuals, and the possibility of drug-resistant viruses emerging if/when the drugs are taken
inconsistently, is known as pre-exposure prophylaxis or PrEP, and was approved by the FDA in 2012 (1, 3).

While blood, semen, and vaginal secretions are clearly capable of transmitting HIV, most other commonly-encountered body materials and fluids are not considered to pose a risk of HIV transmission. Feces, nasal secretions, saliva, sputum, sweat, tears, urine, and vomitus are not considered potentially infectious unless they are visibly bloody (8). In contrast, breastmilk has been implicated in maternal-fetal HIV transmission, which is discussed below. Body fluids that do not transmit HIV may still be capable of transmitting other more hardy viruses such as Hepatitis A, B, or C, so universal precautions are still recommended for all body fluids.

Maternal-fetal transmission of HIV, where the virus passes from the bloodstream of an HIV-infected woman to her infant before birth, or perinatal transmission that occurs during or immediately following birth, is the third means by which HIV can be transmitted (1). Like blood and sexual contact, transmission occurs as a result of HIV gaining access to the baby’s bloodstream and/or lymphatic system, where it encounters susceptible cells and establishes a new infection. Maternal-fetal and perinatal transmission are complex because there are multiple ways in which they can occur.

The rate of transmission in the U.S. of HIV from an untreated HIV+ mother to her infant is 20-25%, or one out of every four or five newborns (1, 3). The good news is that the majority of newborns born to HIV+ mothers (75-80%) do not become infected, even in the absence of treatment of either the mother or the baby. HIV infection can occur in utero, i.e., across the placenta, as some infants are clearly infected and often symptomatic at birth. Transmission also occurs as a result of exposure to the mother’s blood during the birth process, where even in the simplest vaginal delivery, there is ample opportunity for creation of microscopic breaks and tears in an infant’s skin during his or her transit of the birth canal. Delivery by cesarean section does decrease the risk of transmission, but there is still exposure of the infant to the mother’s blood. When infected during delivery, infants have no evidence of HIV at birth, but show evidence of infection in the weeks that follow. And finally, infants who were not infected before or during birth can become infected after birth via breastfeeding, as breastmilk from an infected woman contains viral particles and HIV-infected WBC that will survive in the immature newborn intestinal tract. This mode of maternal-fetal transmission is particularly prominent in developing countries, where the risk of an infant dying due to poor early nutrition and disease if not breastfed may be greater than the risk of transmitting HIV via breastmilk. In 2007, it was estimated that up to 200,000 infants worldwide were being infected with HIV each year as a result of breastfeeding (1).

As mentioned previously and shown in Table I, antiviral therapy of HIV+ pregnant women was successfully introduced in the U.S. in 1994 (3). Treatment with AZT of HIV+ mothers during pregnancy and/or delivery, and of infants during the first six weeks of life (with no breastfeeding) reduced the rate of transmission from 26% to 8%; more recent studies in developing countries using shorter AZT treatments in mothers only, some of whom breastfed their infants, reduced the rate of transmission by one-third to one-half (see http://www/unaids.org). Where it is available, cART is currently being used to treat HIV+ pregnant women, which typically reduces the risk of maternal-fetal HIV transmission to 5% or less (1,3). As noted in Table 1, in 2013, a very unusual case of maternal-fetal transmission and very early cART became public, known as the “Mississippi Baby” (10). The baby girl was documented at birth to have been HIV-infected in utero by an HIV+ mother who was completely
unaware of her own infection until delivery of the baby. The baby was treated unusually early (30 hours after birth) with very aggressive anti-HIV treatment (cART regimen of three drugs, usually reserved for use with adults). Both mother and daughter dropped out of regular medical care 18 months after the child’s birth, and when they returned to medical care, the daughter was approximately 2 years old, and had not been taking any anti-HIV medications since 15-18 months of age. In spite of the interruption of treatment, the child had almost no detectable HIV in her blood and was HIV-antibody negative. At 3 years of age, the girl has been reported to have no detectable HIV viral RNA without taking any antiviral medications for at least 18 months. Future follow up will determine if this is a complete remission of HIV infection as a result of the early and aggressive treatment, which hopefully will result in lifelong control (or possibly even elimination) of HIV infection without any long-term toxicity due to the early aggressive treatment. In circumstances where maternal-fetal transmission cannot be prevented, the “Mississippi Baby” provides a hopeful clue to treatment approaches that might be able to arrest and maybe even reverse HIV infection in newborns.

THE CONTINUUM OF HIV INFECTION

Any discussion of HIV infection and AIDS needs to emphasize that untreated HIV infection is a continuum, of which the serious medical condition known as AIDS is only a small part (Figure 4A). Likewise, it is important to recognize that early diagnosis of HIV infection and successful cART can dramatically alter this continuum (Figure 4B).

![Figure 4: The Continuum of HIV Infection.](image)

A typical continuum is shown for (A) an HIV-infected individual before the introduction of cART, and for (B) a young person (HIV-infected at 20-30 years of age) who receives early HIV diagnosis (DX) and appropriate current cART and HIV-specific medical care throughout his or her lifetime. (Compiled from references 1, 3, 11, 12)
In the first few weeks following HIV infection, a newly-infected person may experience an acute illness with flu-like or mononucleosis-like symptoms such as fever, muscle aches, and headaches (1, 3). In most cases, no serious illness occurs, and within another few weeks, an HIV-infected individual is usually completely asymptomatic. Unless a person knows and/or acknowledges that he or she is at risk for HIV infection, and recognizes the symptoms associated with acute HIV infection, these early signs will pass without notice and without action (i.e., without seeking follow up HIV testing). The asymptomatic phase of the HIV continuum typically lasts for years, during which time the infected person will look and feel well, with no outward sign of his or her HIV infection. In an adult who has not received any kind of anti-viral therapy (e.g., AZT alone in the early days of the epidemic, or cART since 1995), the median time between HIV infection and the development of the clinical diagnosis of AIDS is nine years (Figure 4A) (11). Keep in mind that this is the typical amount of time, but some HIV-infected persons have developed AIDS in the first one or two years after infection while other (rare) individuals continue to be asymptomatic twenty to thirty years after infection without treatment (1, 3). In the years prior to the introduction of cART, or in the case of a person who did not ever receive effective anti-viral treatment, the typical time between a diagnosis of AIDS (now classified by the CDC as Stage 3 HIV disease) and death was/is typically only one to two years (11). Therefore, in most cases even in the absence of treatment, the vast majority of time a person would be expected to spend on the HIV continuum is during the asymptomatic phase, when an individual could be completely unaware of his or her infection. It is extremely important to understand that, although an HIV-infected individual may be asymptomatic for many years (and may or may not know that he/she is infected), HIV-infected persons are infectious as soon as they become infected. In other words, as soon as HIV has successfully established an infection in a person, that person is capable of transmitting the virus to other persons via blood, sexual, or maternal-fetal contact, and such contacts could continue for many years before they themselves become noticeably ill due to their HIV infection.

Regardless of the presence or absence of symptoms, the only routinely available way to determine if a person is infected with HIV is by performing an HIV antibody test. Contrary to what is frequently reported in the popular press, this is not an “AIDS test”, but rather, a test for HIV-specific antibodies which indicate infection with HIV. As with any infection, when HIV successfully infects an individual, the immune system of that individual makes antibodies that will recognize and bind to HIV. These antibodies are intended to neutralize the virus, limit disease, and assist in clearance of the virus from the body. However, because the antibodies are specific to HIV, tests can be designed to detect the HIV-specific antibodies, making them an invaluable tool for diagnosis of HIV infection. Screening HIV antibody tests are based on enzyme-linked immunosorbent assay (ELISA) or other immunoassay (IA) technology, which can be performed manually or with semi- or fully-automated clinical laboratory platforms (Table II) (12). Depending on the test, it can utilize blood serum or plasma, urine, or oral fluids collected with special devices. If a sample is “reactive”, i.e., gives a positive result on an IA, suggesting the presence of HIV-specific antibodies, it is tested a second time by the same method. If “repeatedly-reactive” (positive on both IA tests), the presence of HIV-specific antibodies in the sample is confirmed or ruled out by Western blot (WB) analysis (1, 3, 12). The WB assay is a highly specific but labor-intensive means of detecting specific antibodies. If a sample is reactive on WB with two or more HIV antigens, it is confirmed as truly HIV-antibody positive (HIV+). If a sample is not reactive on WB, or reactive with only one of the HIV antigens, it is considered to
be one of the rare false-positive IA results due to non-specific cross-reactions—such a sample would be reported as HIV-antibody negative (HIV-). Alternatively, a positive IA result can be confirmed by an immunofluorescence assay (IFA). Recommendations for an updated algorithm for diagnostic laboratory HIV testing were drafted in 2012 (Appendix 1), and are to be reviewed in 2014. Hence, it is likely that the testing and confirmation approach outlined above may be revised (and potentially simplified) in the near future. It should be noted that testing for HIV antibody cannot be used in newborn infants, as the tests will pick up maternal antibodies that have crossed the placenta into the baby’s circulation. Therefore, the criteria for diagnosing neonatal HIV infection rely on other types of testing (such as NAT that directly detects the presence of HIV), as well as clinical signs and symptoms (13).

The average time in an adult between HIV infection and the development of detectable HIV antibodies by first, second, or third generation HIV antibody tests is estimated to be 22-25 days (1, 3, 7, 12). In the vast majority of HIV-infected persons, one of these HIV antibody tests will be positive by four to eight weeks after infection (Figure 4A) but in very rare individuals, may take up to 6 months to become positive. This is why, in its recommendations for exposed HCP, the PHS suggests that antibody testing be performed up to six months post-exposure (8). However, with the introduction of more sensitive fourth generation combination tests that detect either HIV antibodies or HIV p24 antigen (Table II), the average time for a first detection of HIV antibodies is estimated to be around 17 days after infection, or slightly less than 3 weeks (Figure 4B). When one of these tests is utilized, four months is considered sufficient time to detect all individuals who are HIV-infected. Whether used in the weeks/months after a known or suspected exposure or many years after high-risk behavior, HIV antibody testing can be an extremely powerful tool in determining whether or not a person is HIV-infected, especially during the asymptomatic period. However, keep in mind that a single negative antibody or even a combination antibody/antigen (ab/ag) test does not guarantee that a person is not infected. As mentioned previously, there is the “window” period during which a person could be HIV-infected, but not yet have enough antibodies and/or p24 antigen to be detected by IA testing.

Individuals who seek HIV testing may not return for test results if a second visit is required. This has led to the development of rapid HIV tests designed for use at point-of-care. Although there are the usual concerns regarding the performance of any laboratory diagnostic test outside of the clinical laboratory, the tremendous benefit of being able to perform on-site tests in order to provide test results and counseling to patients or clients at the same visit clearly justifies FDA-approval of such testing. Rapid HIV tests are particularly valuable in emergency room and community clinic settings. Seven rapid tests for detection of HIV-1 and/or HIV-2 antibodies that produce results in 5 to 20 minutes are licensed for use in the United States (12) (Table II). These rapid tests vary somewhat on the technology used, but all are based on same concept as the traditional immunoassays, i.e., the detection of antibodies in blood or oral fluid that are specifically reactive with HIV. Rapid HIV antibody tests vary in their CLIA status, with either waived or moderate complexity depending on the sample type and the test device used. There is one additional FDA-licensed rapid test which is a fourth generation combination test, similar to the most sophisticated and sensitive clinical laboratory tests (Table II). While it is not quite as sensitive as the automated laboratory-based fourth generation immunoassays, this rapid test yields results in 20 minutes, and is capable of detecting both HIV antibodies and HIV p24 antigen, and also determining if the positive result is due to antibodies, antigen, or both.
It has also been recognized that there may be individuals at risk for HIV infection who will be unwilling to utilize any kind of community- or clinic-based HIV testing service. Therefore, the FDA has approved two home HIV tests that enable someone to initially determine his or her HIV antibody status in private (12). There is a rapid test performed by the user (OraQuick In-Home HIV Test) that utilizes an oral fluid sample (which is not the same as saliva), which provides results in 20 minutes. If positive, a follow-up test through traditional providers is indicated. The other test (Home Access HIV-1 Test System) is a home collection kit, which involves collecting a finger-prick blood sample, sending it to a licensed laboratory, and then calling in for results a few days later. If positive, a follow-up test is performed right away. Both of these tests detect HIV infection later than blood-based diagnostic tests offered by providers, but may facilitate testing among individuals who would otherwise choose to remain ignorant of their HIV status.

The HIV antibody or ab/ag combination tests (and confirmatory WB or IFA) are the only tests approved for diagnosis of HIV infection in adults, and so is the only testing which should be used for that purpose. However, there are a variety of clinical laboratory and/or reference laboratory tests available for evaluating an individual’s immunologic and virologic status throughout the continuum of HIV infection. One very important measurement is the determination of lymphocyte subsets by flow cytometry (2), especially to determine percentage and absolute number of CD4-positive TH cells. The normal range for TH cells is typically 32-60% of total lymphocytes and approximately 500-1600 TH cells/μL of blood (14). However, in HIV infection, it is a loss of TH helper cells which leads to progression of HIV disease through three clinical stages, the last of which is the severe immunodeficiency known as AIDS (1, 3, 13). Therefore, monitoring of TH cell numbers can help to determine how far along the HIV continuum an individual may be and/or how quickly he or she is moving along the continuum.

Since its introduction in the mid-1990s (Table 1), plasma HIV RNA viral load has joined TH cell number as an important indicator of an HIV-infected individual’s status along the HIV continuum (1, 3). HIV viral load measurements determine the amount of extracellular viral RNA present in the circulation and are usually expressed as viral copies per milliliter of plasma. Although the HIV viral load is undetectable for approximately the first ten days of an HIV infection, it peaks during the first weeks after infection, and then falls to some lower level that is usually stable in the absence of anti-viral treatment (1). This stable level or “set point” will differ from person to person, i.e., from undetectable (<50 viral copies/mL in the assay now widely used) to >100,000 copies/mL, but will vary only slightly over time within a single individual. Studies have demonstrated that the lower an individual’s set point, the better the chance that he or she will survive the next 5-10 years without developing AIDS. Therefore, viral load measurements in the absence of or prior to treatment can help to predict the length of time an individual may stay in the asymptomatic portion of the HIV infection continuum. Perhaps more importantly, HIV viral load is critical when assessing and monitoring the efficacy of cART, where the goal is to significantly reduce the viral load (preferably to undetectable levels) and keep it at low or undetectable levels. A rebound of the viral load during cART (e.g., from undetectable to detectable, or a significant increase in detectable viral load) generally indicates development of viral resistance to the drug therapy and/or failure of the infected individual to consistently take the medications. For the quantitation of HIV-1 RNA in plasma of patients, there are three FDA-approved assays based on reverse-transcription polymerase chain reaction (RT-PCR): Amplicor HIV-1 Monitor and COBAS AmpliPrep/COBAS TaqMan from Roche Molecular.
Systems, and Abbot RealTime HIV-1 from Abbott Molecular. There is one additional FDA-approved HIV RNA assay, Versant HIV-1 RNA 3.0, which uses branched DNA (bDNA) signal amplification technology from Siemens Healthcare Diagnostics (formerly known as Bayer, or Chiron). Details on these and all other FDA-approved HIV diagnostic assays can be found at [www.fda.gov](http://www.fda.gov).

In a typical adult without effective anti-retroviral treatment, the asymptomatic phase of the HIV continuum is estimated to last approximately 9 years (Figure 4A). Since there is no outward sign of viral activity during this time, this is often referred to as a period of “clinical latency”. However, in the blood, lymph nodes, and tissues of an HIV-infected person’s body, the virus is anything but latent (1). Even in an individual who has an undetectable HIV viral load, HIV is busy infecting and affecting T<sub>H</sub> cells, monocytes, macrophages, dendritic cells, and brain cells. Therefore, even though an individual is asymptomatic, HIV is slowly damaging the immune system (and in many cases, the brain as well). In particular, HIV infection results in the decline in the number of T<sub>H</sub> cells over time, and the function of the remaining T<sub>H</sub> cells becomes abnormal (1, 3). When the T<sub>H</sub> cells are unable to provide adequate help to B cells and CD8 T cells (Figure 1) due to reduced number and/or function, the immune system becomes unable to prevent disease caused by certain types of pathogens and/or cancers.

As of the latest revision of the case definitions for HIV infection by the CDC in December, 2008, the continuum of HIV infection has been divided into three stages, based on numbers of CD4+ T<sub>H</sub> cells circulating in the blood and the absence or presence of one of the AIDS-defining conditions (Table III) (13). There are 27 different conditions that are considered to be AIDS-defining, i.e., are required for the diagnosis of the most advanced stage (Stage 3/AIDS) of HIV infection. It is the occurrence of any one of these unusual “opportunistic” infections, or cancers associated with immunodeficiency, or a dangerously low T<sub>H</sub> cell count (less than 200 cells/μL), which indicates that an HIV-infected individual has moved into the late, symptomatic phase of HIV disease. While it is still unclear as to exactly how and why infection with HIV has such a dramatic impact on T<sub>H</sub> cells, there is no dispute among mainstream scientists over the fact that HIV infection causes AIDS (1, 3).

There are many additional aspects of HIV infection and AIDS that may be of interest to clinical laboratory scientists. The information covered in this course is intended to provide a review and/or a foundation for understanding HIV and AIDS at a very basic level.
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<table>
<thead>
<tr>
<th>Year</th>
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<tr>
<td>December, 1980</td>
<td>Isolation of first human retrovirus (HTLV-1)</td>
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<tr>
<td>Summer, 1981</td>
<td>First reports of <em>Pneumocystis</em> pneumonia, Kaposi's sarcoma, other unusual infections in homosexual men in Los Angeles, New York, and San Francisco</td>
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<td>1981-82</td>
<td>Similar illnesses reported in injecting drug users, blood transfusion recipients, hemophiliacs; illnesses in homosexual and heterosexual partners, babies of women with immunodeficiency illnesses = ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)</td>
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<td>Late 1983</td>
<td>Isolation of Lymphadenopathy-Associated Virus (LAV), Pasteur Institute, France</td>
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<tr>
<td>Early 1984</td>
<td>Isolation of Human T-Lymphotropic Virus III (HTLV-III), National Institutes of Health, USA; AIDS-Related Virus (ARV), University of California, San Francisco, USA</td>
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<tr>
<td>March, 1985</td>
<td>Introduction of first-generation HTLVIII/LAV (HIV-1) antibody test in U.S. blood banks</td>
</tr>
<tr>
<td>1985-86</td>
<td>Study/approval of AZT (zidovudine, Retrovir) as first drug for treatment of AIDS</td>
</tr>
<tr>
<td>1986</td>
<td>Consensus name agreed upon = HUMAN IMMUNODEFICIENCY VIRUS (HIV-1); related virus identified (HIV-2)</td>
</tr>
<tr>
<td>1993-1995</td>
<td>HIV infection #1 cause of death in U.S. among persons 25-44 years of age</td>
</tr>
<tr>
<td>1994</td>
<td>AZT shown to reduce mother-to-infant HIV transmission during pregnancy and birth</td>
</tr>
<tr>
<td>1995</td>
<td>Introduction of combination anti-retroviral therapy (cART) including protease inhibitors (also known as highly active antiretroviral therapy or HAART); introduction of plasma HIV RNA measurements (HIV viral load)</td>
</tr>
<tr>
<td>1998</td>
<td>63% decrease in U.S. AIDS deaths compared to 1995</td>
</tr>
<tr>
<td>1999</td>
<td>HIV nucleic acid testing (NAT) introduced into U.S. blood banks</td>
</tr>
<tr>
<td>2006</td>
<td>Routine HIV testing recommended by CDC for all adults/adolescents in U.S.</td>
</tr>
<tr>
<td>2009</td>
<td>“Berlin Patient” demonstrates proof-of-principle for functional HIV cure (bone marrow transplant from HLA-matched donor carrying rare HIV-resistant mutation)</td>
</tr>
<tr>
<td>2010</td>
<td>HIV treatment shown to dramatically reduce sexual transmission = “treatment as prevention”; anti-HIV vaginal gel shown to reduce sexual transmission to women</td>
</tr>
<tr>
<td>2010-2013</td>
<td>Fourth-generation combination HIV-1/2 antibody/HIV p24 antigen tests introduced</td>
</tr>
<tr>
<td>2012</td>
<td>World Health Organization/UNAIDS global estimates = 2.3 million new HIV infections and 1.6 million AIDS deaths during 2012, approximately 35 million persons currently living with HIV/AIDS; 52% decrease in new HIV infections in children since 2001</td>
</tr>
<tr>
<td>2013</td>
<td>Report of HIV remission (near complete elimination) in 3 year-old child after maternal-fetal transmission and aggressive early cART 30 hours after birth (“Mississippi Baby”)</td>
</tr>
<tr>
<td>2013</td>
<td>Estimated continued rate of 50,000 new HIV infections/year in U.S.</td>
</tr>
</tbody>
</table>

Compiled from references 1, 3, 4, 9, and additional information available from the Centers for Disease Control and Prevention (www.cdc.gov), and UN Programme on HIV/AIDS (www.unaids.org)
# TABLE II: DIFFERENT TYPES OF FDA-APPROVED HIV IMMUNOASSAYS

<table>
<thead>
<tr>
<th>Generation, type</th>
<th>CLIA Complexity</th>
<th>Immunoassay name(s)</th>
<th>Comments</th>
</tr>
</thead>
</table>
| 2<sup>nd</sup> gen, rapid lateral-flow | Waived (if whole blood or oral fluid) | Clearview HIV1/2 STAT-PAK
Clearview COMPLETE HIV 1/2
OraQuick Advance Rapid HIV-1/2 Ab Test
Uni-Gold Recombigen HIV 1/2 Test | Result <20 min; less sensitive than 3<sup>rd</sup> or 4<sup>th</sup> generation assays |
| 2<sup>nd</sup> gen, rapid flow-through | Waived | INSTI HIV-1 Ab Test | Result <5 min; more sensitive than lateral flow |
| 2<sup>nd</sup> gen, rapid flow-through | Moderate | Reveal G3 Rapid HIV-1 Antibody Test | Result <20 min; differentiates HIV-1 from HIV-2 |
| 2<sup>nd</sup> gen, manual | Moderate | Multispot HIV-1/HIV-2 Rapid Test | |
| 3<sup>rd</sup> gen, fully automated | High | Avioq HIV-1 Microelisa System | Low cost; dried blood spots and oral fluid |
| 3<sup>rd</sup> gen, fully automated | Moderate | ADVIA Centaur HIV 1/0/2 Enhanced (EHIV) | Initial result <1 hour; more sensitive than rapid and 2<sup>nd</sup> gen tests |
| 3<sup>rd</sup> gen, fully automated | High | Orthos Virtos ECI/ECIQ Anti-HIV 1+2 | |
| 4<sup>th</sup> gen, rapid | Moderate | Determine HIV-1/2 Ag/Ab Combo Test | Result 20 min; intermediate sensitivity between 3<sup>rd</sup> – 4<sup>th</sup> gen laboratory assays |
| 4<sup>th</sup> gen, fully automated | Moderate | Abbott Architect HIV Ag/Ab Combo Assay | Initial result <30 min; highly sensitive during early HIV infection |
| 4<sup>th</sup> gen, semi-automated | High | Bio-Rad GS HIV Combo Ag/Ab EIA | >3 hour; highly sensitive during early HIV infection |

Adapted from reference 12 (http://www.cdc.gov/hiv/testing/lab/guidelines/index.html)
TABLE III: Surveillance case definition for human immunodeficiency virus (HIV) infection among adults and adolescents (aged ≥13 years) — United States, 2008

<table>
<thead>
<tr>
<th>Stage</th>
<th>Laboratory evidence*</th>
<th>Clinical evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Laboratory confirmation of HIV infection and CD4+ T&lt;sub&gt;H&lt;/sub&gt; cell count of ≥500 cells/μL or CD4+ T&lt;sub&gt;H&lt;/sub&gt; cell percentage of ≥29</td>
<td>None required (but no AIDS-defining condition)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Laboratory confirmation of HIV infection and CD4+ T&lt;sub&gt;H&lt;/sub&gt; cell count of 200–499 cells/μL or CD4+ T&lt;sub&gt;H&lt;/sub&gt; cell percentage of 14–28</td>
<td>None required (but no AIDS-defining condition)</td>
</tr>
<tr>
<td>Stage 3 (AIDS)</td>
<td>Laboratory confirmation of HIV infection and CD4+ T&lt;sub&gt;H&lt;/sub&gt; cell count of &lt;200 cells/μL or CD4+ T&lt;sub&gt;H&lt;/sub&gt; cell percentage of &lt;14†</td>
<td>or documentation of an AIDS-defining condition (with laboratory confirmation of HIV infection)†</td>
</tr>
<tr>
<td>Stage unknown§</td>
<td>Laboratory confirmation of HIV infection and no information on CD4+ T&lt;sub&gt;H&lt;/sub&gt; cell count or percentage</td>
<td>and no information on presence of AIDS-defining conditions</td>
</tr>
</tbody>
</table>

* The CD4+ T-lymphocyte percentage is the percentage of total lymphocytes. If the CD4+ T-lymphocyte count and percentage do not correspond to the same HIV infection stage, select the more severe stage.

† Documentation of an AIDS-defining condition (Appendix A) supersedes a CD4+ T-lymphocyte count of ≥200 cells/μL and a CD4+ T-lymphocyte percentage of total lymphocytes of ≥14. Definitive diagnostic methods for these conditions are available in Appendix C of the 1993 revised HIV classification system and the expanded AIDS case definition (CDC. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR 1992;41[No. RR-17]) and from the National Notifiable Diseases Surveillance System (available at http://www.cdc.gov/epo/dpshi/casedef/case_definitions.htm).

§ Although cases with no information on CD4+ T-lymphocyte count or percentage or on the presence of AIDS-defining conditions can be classified as stage unknown, every effort should be made to report CD4+ T-lymphocyte counts or percentages and the presence of AIDS-defining conditions at the time of diagnosis. Additional CD4+ T-lymphocyte counts or percentages and any identified AIDS-defining conditions can be reported as recommended. (Council of State and Territorial Epidemiologists. Laboratory reporting of clinical test results indicative of HIV infection: new standards for a new era of surveillance and prevention [Position Statement 04-ID-07]; 2004. Available at http://www.cste.org/ps/2004pdf/04-ID-07-final.pdf).

From reference 13 [MMWR 57 (No. RR-10):4. 2008.]

1. An FDA-approved 4th generation HIV-1/2 immunoassay (IA) should be used as the initial test, to screen for acute HIV-1 infection and for established infections with HIV-1 or HIV-2.
2. Specimens with a reactive 4th generation IA (or repeatedly reactive, if repeat testing is recommended by the manufacturer) should be tested with an FDA-approved 2nd generation antibody IA that differentiates HIV-1 antibodies from HIV-2 antibodies.
3. Persons whose specimens give positive results on the initial IA and HIV-1/HIV-2 antibody differentiation IA should be considered positive for HIV-1 or HIV-2 antibodies and should initiate medical care that includes laboratory tests (such as viral load, CD4 determinations, and antiretroviral resistance assays) to confirm the presence of HIV infection, to stage HIV disease, and to assist in the selection of an initial antiretroviral drug regimen. [DHHS Guidelines]
4. Specimens that are reactive on the initial assay and negative on the HIV-1/HIV-2 antibody differentiation IA should be tested with an FDA-approved nucleic acid test (NAT) for HIV-1 RNA. Under these circumstances, a reactive NAT result indicates the presence of acute HIV-1 infection. A negative result indicates the absence of HIV-1 infection, either a false-positive result on the initial IA or rarely, recent HIV-2 infection. If HIV-2 infection is a possibility, a NAT for HIV-2 DNA can be considered. However, HIV-2 infection is rare in the United States, and there is no FDA-approved NAT for HIV-2.
5. This same testing algorithm beginning with a 4th generation immunoassay should be followed for specimens from persons with a preliminary positive rapid HIV test result.

How these recommendations differ from previous recommendations
2. Tests for both virologic (p24 antigen) and serologic (antibody) markers of HIV infection
3. Incorporates NAT to resolve discordant IA results, reduce indeterminate test results, and identify acute HIV infection
4. All antibody-positive specimens tested for HIV-2; previously, only those with negative or indeterminate HIV-1 Western blot received specific HIV-2 testing.
5. Emphasizes sensitivity during initial testing. Rare false-positive antibody test results might occur; will be resolved during subsequent laboratory testing (e.g., HIV viral load) recommended as part of initial clinical evaluation.

From reference 3 (http://www.cdc.gov/hiv/pdf/policies_Draft_HIV_Testing_Alg_Rec_508.2.pdf)
REVIEW QUESTIONS
Course # DL-968
Choose the one best answer

1. In order to mount antigen-specific secreted antibody responses, B cells require:
   a. interactions with monocytes
   b. cell-to-cell contact with TH cells
   c. intracellular infection of target cells
   d. Only secreted cytokines from TH cells

2. The CD4 molecule is found on:
   a. TH cells
   b. CTL-P cells
   c. B cells
   d. Colon-Diverticular cells

3. IL-2 produced by TH cells:
   a. drives CD4 T cells to proliferate and become plasma cells
   b. signals the TH cells that produced it to differentiate into a stem cell
   c. provides help for CTL-P to mature into functional CTL capable of killing
   d. acts only during cell-to-cell contact with another TH cell

4. TH cells are critical for:
   a. antigen-specific cell-mediated responses only
   b. non-specific phagocytosis in inflammatory responses
   c. both humoral and cell-mediated antigen-specific immune responses
   d. antigen presentation in B cell activation

5. The first human retrovirus successfully isolated in the laboratory was:
   a. HIV-2
   b. HTLV-II
   c. HIV-1
   d. HTLV-I

6. The first cases of what came to be known as AIDS (recognized due to Pneumocystis pneumonia and Kaposi’s sarcoma) were reported in the U.S. in:
   a. 1981
   b. 1985
   c. 1991
   d. 1995

7. First-generation antibody testing for HIV-1 was introduced into U.S. blood banks in:
   a. 1981
   b. 1985
   c. 1991
   d. 1999
8. Nucleic Acid Testing (NAT) is utilized in blood banks to:
   a. discriminate between antibodies specific for HIV-1 and HIV-2
   b. replace the confirmatory Western blot assay
   c. screen for infection with more than five different blood-borne human viruses
   d. provide earlier detection of recently HIV-infected donors compared to HIV-antibody testing alone

9. By 1998, there was a >60% decline in the number of AIDS-related deaths in the U.S. compared to 1995. These declines are attributed to:
   a. an increase in federal funding for clean needle and condom distributions
   b. the introduction of federal policies mandating access to medical care for persons with AIDS
   c. the introduction of multi-drug therapy including a new class of antiretroviral drugs known as protease inhibitors
   d. changes in CDC requirements for the reporting of AIDS cases

10. In 2006, HIV antibody testing was recommended routinely by the CDC for:
    a. only those pregnant women with documented HIV+ sexual partners
    b. all adults and adolescents ≥13 years of age
    c. newborn infants born to HIV+ mothers
    d. all travelers arriving from HIV-endemic areas

11. cART is an acronym for:
    a. “combination anti-retroviral therapy”
    b. “cellular activation response of T-cells”
    c. “chemically-augmented reverse transcription”
    d. “chronic AIDS-related transmission”

12. Cells that can be infected by HIV include:
    a. T<sub>H</sub> cells, monocytes, and glial cells
    b. B cells and T cells
    c. CTL-P cells
    d. T cells, macrophages, and skin cells

13. The unique genetic process that distinguishes retroviruses is called:
    a. reverse translation
    b. reverse transcription
    c. post-translation modification
    d. DNA transcription

14. HIV is not transmitted by:
    a. sexual contact
    b. blood contact
    c. casual contact
    d. breastfeeding
15. Establishment of a new HIV infection by HIV-infected WBCs can occur via:
   a. cleansing of the skin with rubbing alcohol
   b. cell-to-cell contact with CTLs
   c. digestion within the intestinal tract
   d. gaining access to the lymphatic system

16. The addition of NAT in blood bank screening has reduced the period during which a donor might be HIV-infected but not be detected by the screening tests:
   a. from 6 months to 4-6 weeks
   b. from 8-10 years to 6 months
   c. from approximately 3 weeks to 7-10 days
   d. to 5 days

17. The estimated risk of HIV infection following a needlestick exposure to blood from a known HIV-infected individual is:
   a. Less than 1 in 1,000
   b. 1 in 1,000
   c. 1 in 20
   d. 1 in 200 to 1 in 500

18. In the absence of visible blood, the body fluid that should be considered to pose a risk for occupationally-acquired HIV infection is:
   a. urine
   b. nasal secretions
   c. semen
   d. vomitus

19. The best protection against occupational exposure to HIV for clinical laboratory scientists is:
   a. Standard Precautions
   b. universal HIV-antibody testing of patients
   c. screening patients for HIV infection as a requirement for hospital admission
   d. manual recapping of all used needles/syringes

20. It is the recommendation of the PHS that any HCP exposed to blood or other potentially infectious materials be:
   a. referred to counseling at least 72 hours after the possible HIV exposure
   b. evaluated as soon as possible for timely initiation of HIV PEP
   c. scheduled for treatment consultation during the next monthly infectious disease conference
   d. denied medical services due to potential legal liability in the absence of post-exposure protocols

21. Which of the following is not recommended by PHS as an element of appropriate occupational post-exposure management:
   a. immediate cleansing of exposed skin with soap and water or flushing of mucous membranes with water
b. discontinuation of work practice controls
c. diagnostic HIV testing on source person in compliance with all applicable laws
d. pregnancy testing in female HDCPs who have been potentially exposed

22. A baseline blood sample from a potentially-exposed HCP:
   a. is not recommended
   b. should be obtained and tested for HIV antibodies with his/her consent
   c. is only for the protection of the institution in the event of an occupationally-acquired infection
   d. should be obtained and tested for HIV antibodies only if PEP is initiated

23. HIV cannot be transmitted from an HIV-infected mother to her infant:
   a. by cuddling
   b. through breastfeeding
   c. as a result of blood exposure during birth
   d. across the placenta

24. HIV infection:
   a. is indistinguishable from AIDS
   b. never causes an acute illness
   c. is transmitted only if the infected individual is aware of his/her HIV status
   d. is a continuum during which the majority of time is spent in an asymptomatic phase

25. A positive result on an HIV antibody IA:
   a. is valid on a newborn infant born to an HIV+ mother
   b. cannot ever be reported if obtained on a urine sample
   c. can be disregarded in the absence of HIV-related symptoms
   d. must be confirmed as positive by WB or IFA

26. Jane Doe had a one-time sexual contact with a known HIV-infected man on the evening of December 31, 2013. Jane Doe then had a third-generation HIV antibody test performed on January 10, 2014, which gave a negative result. This result means:
   a. Jane Doe cannot possibly be infected because she has no symptoms yet
   b. Jane Doe is definitely not infected as a result of the December 31st exposure
   c. Jane Doe may or may not be infected—it is too soon to rule out a December 31st infection
   d. Jane Doe may be infected, but it will be 9 years before her infection can be diagnosed

27. HIV viral load measurements determine:
   a. the amount of HIV viral RNA present in the circulation
   b. the number of HIV-infected cells in the body
   c. the length of time a person has been infected
   d. the amount of virus to which a person has been exposed
28. None of the rapid HIV-antibody tests are:
   a. CLIA-waived tests
   b. as sensitive as 4th generation ab/ag laboratory tests
   c. able to provide results in less than 5 minutes
   d. designed to be used with oral fluid

29. For a young adult in the U.S. who becomes HIV-infected before the age of 30, and receives prompt HIV-specific medical care including cART, he or she could expect to live:
   a. 1-2 years
   b. approximately 9 years
   c. >50 years
   d. 5-8 years

30. HIV infection progresses to Stage 3 (AIDS) because:
   a. HIV causes a decrease in the number and function of $T_H$ cells
   b. HIV depletes the body of macrophages that act as a reservoir
   c. HIV activates all types of lymphocytes, increasing the number of cells in the circulation
   d. HIV selectively infects only men who have sex with men