*Clinical Chemistry* Trainee Council
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**TITLE: HIV Serology Testing**

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**Slide 1:**
Hello, my name is Hui Chen. I am a former Molecular Genetic Pathology fellow at Memorial Sloan-Kettering Cancer Center. My co-presenter, Yi-Wei Tang, is the chief of Clinical Microbiology Service at the Memorial Sloan-Kettering Cancer Center. Welcome to this Pearl of Laboratory Medicine on "HIV Serology Testing."

The human immunodeficiency virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS). Worldwide, 34 million people are living with HIV infection and 1.7 million deaths due to AIDS in 2011.The U.S. Centers for Disease Control and Prevention (CDC) estimates that approximately 50,000 people are infected with HIV each year. Serology has been the mainstay for HIV infection diagnosis in the clinical laboratories.

**Slide 2:**
HIV is a member of the lentivirus subfamily of the Retroviridae. It is an enveloped virus that contains a capsid enclosing single stranded RNA dimer. Replication of HIV proceeds with reverse transcription of genomic RNA into DNA intermediate, which is integrated into host cell genome. HIV replicates in an error prone manner that generates a mutation virtually every time it replicates. Major gene products of diagnostic significance for HIV-1 include envelope proteins (gp160, gp120, gp41), the capsid proteins (p55, p24, p17), and polymerase gene proteins (p66, p51, p31).

**Slide 3:**
The objectives for HIV serology testing are confirmation that an individual who has presented with signs or symptoms compatible with HIV infection is truly infected, and determination of the HIV status of an asymptomatic person who may have been exposed to HIV. Initial screening tests must be selected carefully, and if reactive, followed by confirmation assays to rule out false-positive results and to verify true HIV infection. HIV screening tests include rapid tests and enzyme immunoassay (EIA). The confirmation assays include multispot HIV-1/HIV-2 rapid test in conjunction with fourth-generation EIA in the new HIV test algorithm, and Western blot assay and immunofluorescence assay (IFA) in traditional algorithm. Detuned assays differentiate recent versus established infections.

**Slide 4:**

In contrast to conventional HIV enzyme immunoassays which are associated with high rates of non-return for test results, rapid HIV antibody tests approved by the U.S. Food and Drug Administration (FDA) reduce unrecognized infections by improving access to testing in both clinical and nonclinical settings and increase the proportion of patients tested who learn their results. The tests are performed when immediate actions are needed in situations of perinatal and occupational-exposure. Rapid HIV tests are simple to use with minimal equipment needed. The test can be performed by any health care worker with basic training. Test results are interpreted visually at the end of the reaction. Currently, seven rapid HIV tests approved by the FDA are commercially available for use in the United States. These tests include OraQuick advance rapid HIV-1/2 Antibody test, Uni-Gold Recombigen HIV test, Reveal G-3 rapid HIV-1 antibody test, Multi Spot HIV-1/HIV-2 rapid test, Clearview HIV 1/2 STAT-PAK, Clearview COMPLETE HIV1/2, and INSTI HIV-1 antibody test. Only OraQuick advance rapid HIV1/2 antibody test can be performed in home and over-the-counter settings.

**Slide 5:**

OraQuick advance rapid HIV1/2 antibody test is one of the CLIA-waived rapid screening tests for use with fingerstick, venipuncture blood and plasma, and oral fluid. The kits are stored at room temperature. The device contains a nitrocellulose strip with synthetic gp41 peptides representing the HIV-1 envelope and the gp36 region of the HIV-2 envelope applied in the “T” (test) location, and a stripe of goat antihuman IgG in the “C” (control) location. Thus, the test screens for both HIV-1 and HIV-2. If HIV antibodies are present in the specimen, they bind to the peptides causing a read line to appear in the test location. The red line in control location indicates a valid test result. Test results should be read no sooner than 20 minutes and no later than 40 minutes after the test device is inserted into the developer vial.

**Slide 6:**

Conventional enzyme immunoassays (EIA) are initial screening tests. Most assays use purified antigen produced either from viral lysates or recombinant or synthetic peptides for detection of HIV-1 and HIV-2 antibodies. The average seroconversion (production of specific antibody to HIV) is about 21 days; thus, the antibody tests for HIV do not detect acute HIV infection. Improvement of initial tests over time have led to the designation of test methods as first-, second-, third-, and fourth-generations. The first-generation immunoassays use viral lysate antigens. The test is less sensitive and is associated with more false positive results. The third-generation immunoassays use recombinant antigens and give less false positive results. The major improvement is the fourth-generation immunoassays, which combine third-generation antibody detection with HIV p24 Ag detection, allowing for detection of both antibodies and antigens simultaneously. In the new HIV diagnostic algorithm, reactive results from fourth-generation immunoassay can be confirmed by multispot HIV-1/HIV-2 rapid test, and no need of Western blot confirmation. Thus, the turn-around-time is improved. The sensitivity of third- and fourth-generation immunoassay is approximately 98-100% with fourth-generation being more sensitive than third-generation. The specificity of third- and fourth-generation is approximately 99.8%.

**Slide 7:**

Clinically, HIV infection progresses through stages from acute HIV infection, to asymptomatic or “latent” phase, and finally full-fledged acquired immunodeficiency syndrome (AIDS). The acute HIV infection is associated with high levels of viremia, generally resolves within a few weeks, with the onset of a cellular

and humoral immune response to HIV. The viremia declines from the peak observed during acute infection and remains detectable over time with the exception of rare population, long-term nonprogressors, also known as elite controllers. Each stage has characteristic laboratory findings. Capsid protein p24, the most abundant viral protein, can be detected transiently within the first few weeks after infection. The disappearance of p24 antigen with seroconversion may be partially due to blocking of p24 detection by the formation of immune complexes with anti-24 antibody. Detection of anti-HIV antibodies is the mainstay of diagnosis for HIV infection. Immunoglobulin M (IgM) antibodies to HIV appear first and transient. The IgM response is followed by a long-lived immunoglobulin G (IgG) response. The period of time from infection until antibody seroconversion (known as the preseroconversion window period) varies from person to person but not depending on the assay. Detection of HIV antibodies and seroconversion varies depending on the assay. The most sensitive fourth-generation combination assays are generally positive four to seven days after detectable virus by nucleic acid testing. The third-generation enzyme immunoassays are generally positive seven to fourteen days after viral RNA is first detected. It takes longer (15 to 30 days) for less sensitive first-generation immunoassay to become reactive, or for the development of a sufficient number of bands on Western blot to meet diagnostic criteria.

**Slide 8:**

The first-generation immunoassay was indirect EIA. These formats used coated (or immobilized) viral lysate antigen on a solid phase for antibody capture and detected antibody using an antihuman IgG conjugate. The second-generation immunoassay used recombinant or synthetic peptide antigen to increase assay sensitivity and specificity. Both first- and second-generation tests can only detect anti-HIV IgG antibody developed relatively late in infection.

**Slide 9:**

The third-generation assays were developed using antigen sandwich principle. The immobilized antigen captured HIV IgM and IgG antibodies in the patient sample. The captured antibodies were detected through a second labeled HIV antigen and subsequent addition of substrate. This allowed for the detection of specific HIV IgM antibody that occurs early in infection, in addition to IgG antibody, which appears later. Thus, third-generation test provided increased seroconversion sensitivity.

**Slide 10:**

The fourth-generation immunoassays incorporated HIV p24 antigen detection into third-generation antibody detection. The solid matrix was immobilized with both HIV antigen and p24 antibody for capturing HIV IgM and IgG antibodies and p24 antigen in the patient sample. The captured antibodies were detected through a second labeled HIV antigen and subsequent addition of substrate. The capture p24 antigen was detected through a second labeled anti-p24 antibody and subsequent addition of substrate. The introduction of p24 antigen detection allowed for the detection of acute HIV infection, and provided the highest seroconversion sensitivity among four generations of EIA.

**Slide 11:**

Until recently, the Western blot assay has been historically referred as the “gold standard” for HIV diagnosis, and used in many algorithms for confirming HIV infection. Although highly specific, the assay

requires time-consuming procedure and thus long turn-around time to report results. In brief, viral lysate antigens were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by their molecular weights, then transferred to nitrocellulose membrane, and blocked with protein-rich solution. This membrane is subsequently cut into strips and used as solid support for EIA testing for anti-HIV antibodies in patient specimen. The test may yield nonspecific reactivity with non-viral proteins. The criterion for positive interpretation requires the presence of any two or more bands of p24, gp41, or gp120/gp160, following the recommendations of the CDC and the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD). The test is interpreted as negative if no band is present and indeterminate if any bands are present but the pattern does not meet the criteria for either positive or negative. Indeterminate Western blot occurs at a rate of 9% with p24 and 3% with gp120/160 in HIV-negative patients. Risk factors are used to determine the frequency of testing and the likelihood of "indeterminate" Western blot pattern to represent HIV infection.

**Slide 12:**

Multispot HIV-1/HIV-2 rapid test is a moderate complexity assay approved by the FDA for use on fresh or frozen serum and plasma to both detect and distinguish HIV-1 from HIV-2. The test cartridge contains a membrane with microparticles immobilized in four spots. Two spots consist of recombinant and synthetic gp41 peptides to detect HIV-1 antibodies; one consists of synthetic gp36 peptides to detect antibodies to HIV-2; and the fourth spot consists of goat antihuman IgG as the internal control. The test is considered positive for HIV-1 if the control spot and both of the HIV-1 spots turn purple, indeterminate for HIV-1 if the control spot and one of the HIV-1 spots turn purple, and positive for HIV-2 if the control and HIV-2 spots appear. If purple appears in the control spot, the HIV-2 spot, and one or both of the HVI-1 spots, the test is considered HIV reactive (undifferentiated). In this case, the specimen may be tested by additional methods which allow differentiation between HIV-1 and HIV-2. The test is negative when only the control spot appears. The absence of control spot indicates an invalid result, regardless of any other spot pattern. The multispot test can be used as confirmation test for reactive result by fourth-generation EIA test in the new HIV test algorithm.

**Slide 13:**

Differentiating individuals with recent HIV-1 infection from those infected for longer periods is important for estimating HIV incidence and for purposes of clinical care and prevention. The discrimination is based on either antibody titer or antibody avidity using modification of current available EIA assay on seroconverted specimen. This has been mainly used for epidemiology purpose when paired serum specimens are not available.

**Slide 14:**

The sensitive/less-sensitive serologic strategy (the serologic testing algorithm for recent HIV seroconversion [STARHS]) uses sensitive ELISA on original sample and less-sensitive ELISA on diluted sample from the patient. The standardized optical density was calculated to estimate the probability of recent seroconverters.

The antibody avidity assay tests for the stability of antibody binding in the presence or absence of a chaotropic reagent (guanidine hydrochloride). In avidity method, two aliquots of each sample are subjected to a pretest 1:10 dilution in either 1M phosphate-buffered saline (PBS) or 1M guanidine

hydrochloride, and tested by immunoassay. The avidity index of HIV antibodies are calculated from the ratio of the two measurements. An avidity index less than cutoff indicates a recent infection.

**Slide 15:**

Lastly, an HIV test algorithm is a step-by-step testing plan to determine the presence or absence of HIV infection for patient screening or diagnosis. The traditional algorithm uses a conventional EIA or rapid test for HIV antibody followed by a more specific supplemental test, either Western blot, immunofluorescent assay, line immunoassay, or qualitative RNA test. Many initial assays are more sensitive during early seroconversion than the traditional supplemental assay, Western blot, and immunofluorescent assay, which produce an increased number of indeterminate or false-negative supplemental tests.

The new HIV diagnostic algorithm overcomes the above limitation. The new algorithm is designed to use a sensitive initial screening assay capable of detecting HIV-1 and HIV-2 infections during the acute, recency, and longstanding phases of infection (optimally, an Ag/Ab combination assay such as fourth-generation assay). Specimens reactive by an initial screening assay should then be tested by an antibody immunoassay able to distinguish HIV-1 from HIV-2 infections (e.g. Multispot HIV-1/HIV-2 rapid test). If the differentiation assay is also reactive, the presence of HIV-1 or HIV-2 antibodies has been established. If the differentiation assay is negative or inconclusive, further testing with nucleic acid testing (NAT) should be performed to identify or rule out acute HIV-1 infection.

In conclusion, HIV is a causative agent for AIDS. The HIV serology tests include initial screening test followed by confirmation assay. The detuned assays can differentiate recent versus established infection.

**Slide 16: References**

**Slide 17: Disclosures**

**Slide 18: Thank You from [www.TraineeCouncil.org](http://www.TraineeCouncil.org)**

Thank you for joining me on this Pearl of Laboratory Medicine on “HIV Serology Testing.” My name is Hui Chen.