

Communiqué

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Feature

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Laboratory Testing*

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HIV/AIDS and Laboratory Testing

Human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) is no longer on the front page of every newspaper. It rarely leads on the nightly news anymore. Despite antiretroviral therapy, interferon therapy, and drug cocktails and with new treatments and vaccines in development, HIV/AIDS is still a significant problem in our society. Additionally, there is a disturbing overall increase in sexually transmitted diseases nationwide. This trend is alarming because people infected with other sexually transmitted diseases are at higher risk of contracting HIV infection, and the trend demonstrates that many people are not following safe sex practices.^{1,2} It is estimated that as many as one third of the HIV-infected population is unaware of their HIV status and are, therefore, at high risk of transmitting the virus to uninfected persons.³

Internationally, it is estimated that over 40 million people are living with HIV/AIDS, and AIDS deaths for 2001 are estimated at 3 million men, women, and children.⁴ While the picture is much more severe for Africa and Asia, the rest of the world cannot assume this health threat is under control. At the end of 2001, there were over 362,000 persons living with AIDS in the United States.⁴ Of the over 816,000 (cumulative) persons reported as having AIDS in the United States, over 467,000 have died.⁴

With the introduction of highly active antiretroviral therapy (HAART) in 1995, the close relationship between the numbers of HIV infections and incidence of AIDS has been dissociated, as the use of HAART brought about significant reductions in the morbidity and mortality of infected patients and slowed the progression to AIDS.⁵ While AIDS incidence in the United States declined dramatically from 1996 to 1999, the rate of decline has now slowed, and the level remained stable through 2001.⁶

There is no cure for HIV/AIDS. Once a patient is infected, detection and monitoring to prevent

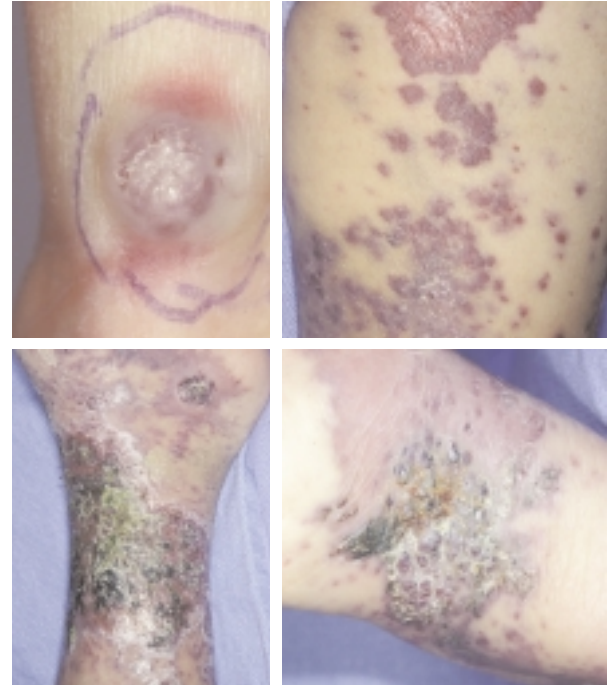


Figure 1. Kaposi's sarcoma, seen rarely prior to the AIDS epidemic, is now recognized as one of the complications of HIV infection. Infected patients also are increased risk of cervical cancer and lymphoma.

unknowing transmission of the virus to uninfected individuals remain the primary weapons in the battle against HIV/AIDS. Patients must also be aware that being infected with HIV puts them at greater risk of coinfection and certain cancers. (See Figure 1.) As a result, when a patient is positive for HIV, additional testing for other sexually transmitted, opportunistic, or infectious diseases is recommended. Current recommendations include testing all HIV-infected persons for tuberculosis and hepatitis C virus infection.^{7,8}

A relatively new field of testing, genotypic analysis for drug resistance mutations, can provide critical information for directing

treatment in patients with known HIV infection. When combination therapy fails, changes in antiretroviral therapy guided by the use of HIV genotypic analysis can result in additional, if short-term, benefit.

Human Immunodeficiency Virus

Human immunodeficiency virus type 1 (HIV-1) is the major etiologic agent of AIDS. The virus is transmitted through sexual contact, by exposure to blood (including sharing contaminated needles and syringes) or certain blood products, or from an infected mother to her fetus or child during the perinatal period.

CD4 lymphocytes (commonly referred to as T-helper cells) coordinate the human body's defense mechanisms including white blood cells and antibodies to attack foreign organisms. HIV, an RNA virus, is able to overcome these defenses, infecting the CD4 lymphocytes. Within the infected cell, HIV viral reverse transcriptase (RT) enzyme is produced to generate complementary DNA (cDNA) from the viral RNA template. RT has little proofreading capacity and, therefore, incorporates errors in the proviral DNA, causing the mutations that could be associated with resistance. These errors are transcribed into infectious viral particles when the proviral DNA is transcribed into RNA. Similarly, the viral enzyme protease catalyzes a polyprotein to produce peptides necessary for active viral replication. The cycle of infection and replication repeats itself, with viral particles breaking out of the CD4 cells and spreading through the body. Viral production can reach as high as 10 billion HIV particles each day, while the immune system produces up to 2 billion CD4 cells in response. Eventually the virus overwhelms the immune system and the patient develops severe immunodeficiency.

Epidemiologic data suggests that AIDS is caused by at least 2 types of HIV. The first virus, HIV-1 has been isolated from patients with AIDS, AIDS-related complex, and asymptomatic infected individuals at high risk for AIDS. A second HIV virus, HIV-2, was isolated from patients in West Africa in 1986. HIV-2 seems to be endemic only in West Africa, but has also been identified in individuals who have lived in West Africa or had sexual relations with individuals from that region. In 1988, the first case of AIDS in a patient with HIV-2 was reported in the United States. HIV-2 infection in the United States is rarely seen, with less than 100 cases reported to date. HIV-2 is similar to HIV-1 in its morphology, overall genomic structure, and its ability to cause AIDS.

Antibody against HIV-1/2 is usually not detectable until 6-12 weeks following exposure. However, such antibodies are almost always detectable by 12 months. During the course of HIV-1 infection, human antibodies are formed against viral proteins including:

- Envelope: gp41, gp120, gp160
- Core: p17, p24, p55
- Polymerase: p31, p51, p66

Immunosuppressed patients or immunoincompetent patients may fail to develop these antibodies, and antibody to HIV-1/2 may fall to undetectable levels in the terminal stage of AIDS.

Diagnostic Testing

The simplest approach to the diagnosis of HIV infection is to order Mayo Medical Laboratories' (MML) [#9333 Human Immunodeficiency Virus 1/2 Antibody Evaluation](#). This evaluation is designed to provide all the necessary serologic tests, performed in the proper sequence, and includes screening tests that can be followed with confirmatory and exclusionary testing for both HIV-1 and HIV-2.

When the comprehensive evaluation is not a practical option, MML offers individual HIV tests. A "*HIV Testing Algorithm*" is included to help clarify our recommendations for laboratory testing for HIV. (See Figure 2.)

Screening tests are the first step in diagnosis of HIV infection. Simple, relatively inexpensive, US Food and Drug Administration (FDA)-approved blood tests are utilized to screen for the presence of antibodies to the virus. The enzyme immunoassay (EIA), [#9189 Human Immunodeficiency Virus 1/2 Antibody \(combined assay\), Serum](#), is a sensitive and specific test. While the high sensitivity of the test results in some false positives, fewer true positives will go undetected. Sensitivity is most important in screening tests, because if infected persons believe they are uninfected, they could spread the virus to others. Conversely, the extremely negative social, psychological, and financial impact of a positive HIV result on the patient must also be addressed. With the relatively low prevalence of HIV infection in the North American population, the positive predictive value of the EIA test is low. Therefore, all specimens testing positive initially by the EIA screening test are routinely repeated at least once to minimize the risk of a false-positive result. In addition, positive EIA results are confirmed by Western blot testing.

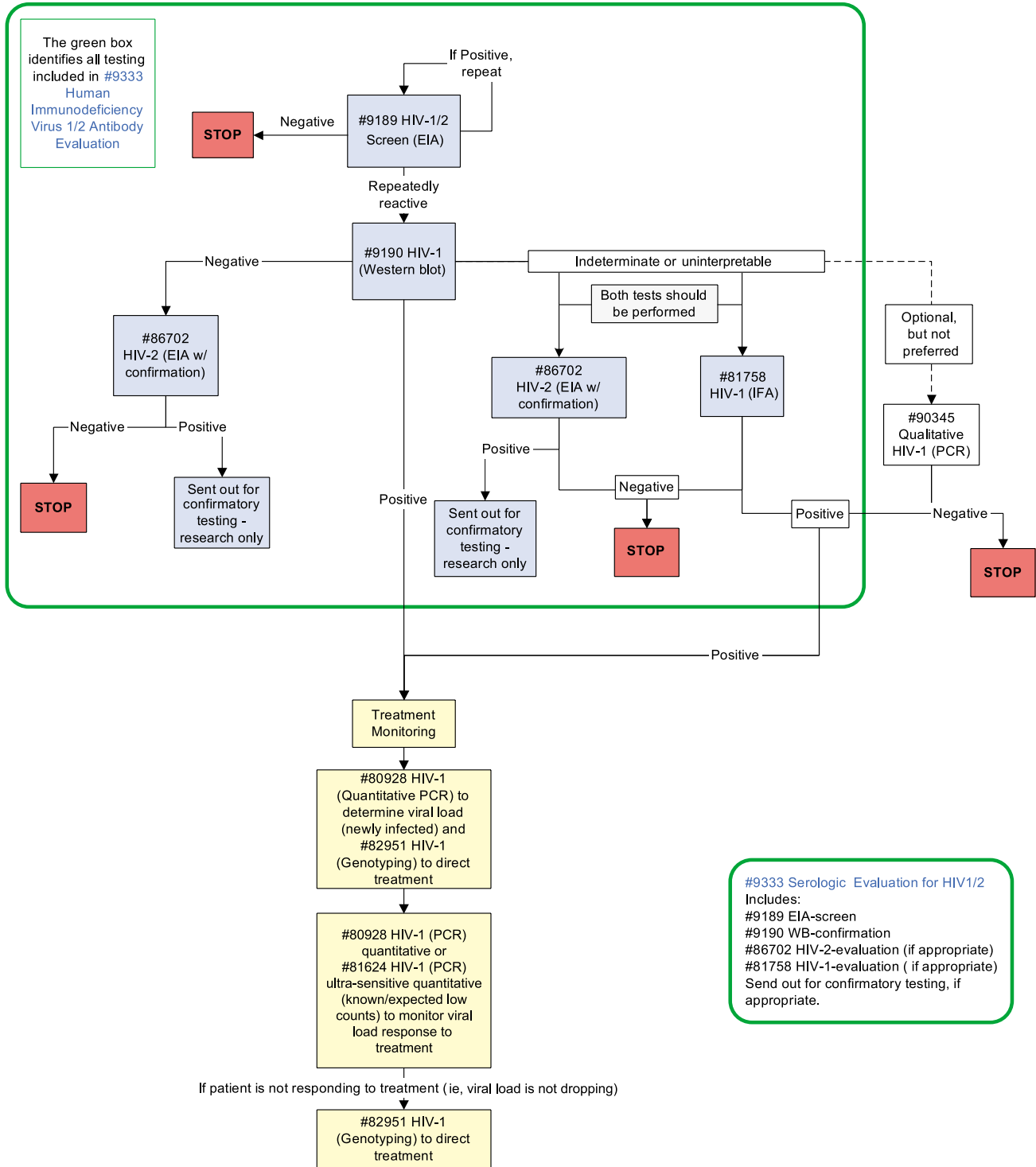


Figure 2. HIV Testing Algorithm

Western blot testing, #9190 Human Immunodeficiency Virus-1 (HIV-1) Antibody, Western Blot Assay, Serum, detects the presence of human antibodies to various HIV-1 proteins. The Western blot test is highly specific for these anti-HIV-1 antibodies and will not react with the non-anti-HIV antibodies that may cause false-positive EIA results. Western blot testing is not appropriate as a screening test because it is more expensive, more labor intensive, frequently yields indeterminate or uninterpretable results, and takes longer to perform than EIA. A patient who has 2 positive EIA screen results and a positive Western blot result is classified as having HIV-1.

Patients with positive HIV 1/2 EIA but indeterminate or negative results on HIV-1 Western blot (especially those that react with core and polymerase proteins only) should be investigated for the presence of antibodies to HIV-2. **#86702 Human Immunodeficiency Virus Antibody Type 2 (Anti-HIV-2), Serum** is available to screen for the presence of HIV-2 proteins. This screening test is usually performed to rule out the presence of HIV-2 simultaneously with the indirect immunofluorescence test (**#81758**) that is performed to rule out HIV-1. An **HIV-2 Western blot** test is performed to confirm the presence of HIV-2 proteins. *HIV-2 Western blot testing is offered for research only, and results are not to be used for diagnostic purposes.*

Additional confirmatory testing for patients with positive EIA and indeterminate Western blot results also is available in an **indirect immunofluorescence (IFA)** assay for HIV-1, **#81758 Human Immunodeficiency Virus (HIV) Type 1 Antibody, Immunofluorescence Assay.** (The evaluation panel [**#9333**] performs both the HIV-1 IFA and the HIV-2 EIA with Western blot, if indicated.) Based upon patient history and other serologic findings, physicians may choose to use the IFA test before proceeding to a molecular qualitative or quantitative HIV-1 test.

Monitoring

Monitoring of the patient's viral load is performed to track disease progression and treatment response. Qualitative and quantitative testing are used to verify the presence and/or level of viral load, respectively.

Quantitative testing is available in 2 different linear ranges: 400-750,000 copies/mL (standard) and 50-100,000 copies/mL (ultra-sensitive). The standard quantitative test, **#80928 Human Immunodeficiency**

Virus Type 1 (HIV-1) RNA by Polymerase Chain Reaction (PCR), Quantitative, Plasma, is routinely used in situations of newly diagnosed HIV infection, as such patients routinely have high viral loads. The test also is useful for monitoring response to treatment.

The ultra-sensitive quantitative test, **#81624 Human Immunodeficiency Virus Type 1 (HIV-1) RNA by Ultra-Sensitive Polymerase Chain Reaction (PCR), Quantitative, Plasma,** is most commonly utilized when the patient is undergoing and responding to HAART therapy. This test is available for quantifying viral load when the patients are known/expected to have very low copy numbers of HIV in their plasma (<100,000 copies/mL), as low as 50 copies/mL. As anti-HIV therapy has become more potent, lower sensitivity requirements for quantitative assays have become necessary. This assay is the most sensitive quantitative HIV assay available at MML.

When the patient's viral load drops below 50 copies/mL, the **qualitative test #90345 HIV-1 DNA, PCR** can be utilized to verify the presence of HIV-1 proviral DNA. However, this test is not approved by the FDA and is intended for *research use only*. The test is neither a screening nor a primary diagnostic test. While available, this is not a commonly ordered test at Mayo. Most cases of HIV-1 infection can be properly diagnosed utilizing serologic testing. The qualitative assay may be useful when the viral load is below the lower limit of detection of the quantitative assays, to resolve discrepant HIV-1 serologic test results, or in determining diagnosis of HIV infection in neonates born to HIV-infected mothers. This qualitative assay does not detect HIV-2.

Genotyping

While patients may be able to extend their life using HAART - utilizing a combination of nucleoside analog, nonnucleoside agent and/or protease inhibitor, and in many cases improve the quality of that time, the therapy is expensive, costing as much as \$1,000/month. In addition, the regimens are complex and require a commitment from the patient to optimize treatment. Although HAART may be effective in reducing the viral load or titer of HIV, antiviral resistance may compromise this therapy. When HAART therapy fails, (demonstrated by rising viral load) genotyping (**#82951 Human Immunodeficiency Virus Type 1 (HIV-1) Genotyping, Plasma**) may be utilized to identify key HIV-1 mutations that are associated with drug resistance.

Sequence analysis of the amplified viral gene targets (protease genes) allows identification of nucleotide changes (mutations) associated with HIV drug resistance. Comparison of sequence data of a patient's viral strain with those in a database developed by the test manufacturer allows identification of key mutations to serve as a guide for initiating, continuing, or changing antiviral treatment regimens.

Antiretroviral therapy prior to 1996 included 4 drugs. In 2002, there are 16 drugs approved for the treatment of HIV infection. Finding the combination that works with each patient requires close monitoring by their physician. Viral load, and in some instances, genotyping should be determined prior to initiating therapy, utilizing the quantitative (#80928) and genotyping (#82951) assays. Once treatment is initiated, the patient's response to therapy is gauged by the patient's viral load. The patient should then be retested every 3-4 months, utilizing either the quantitative assay #80928 or #81624, based upon the previous viral load results.

Summary

MML offers a comprehensive test menu for thorough evaluation and follow-up of the patient suspected or known to be infected with HIV. If you have questions about the appropriate test to order for your patient, our laboratory and consulting staff are available to assist you. Contact Mayo Laboratory Inquiry (MLI) at 800-533-1710.

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JC/BK Virus by PCR Specimen Update

MML has validated 2 additional specimen types for #80067 JC/BK Virus Detection by Polymerase Chain Reaction (PCR), plasma and urine. Previously this assay was only available for use on cerebrospinal fluid. The preferred transport temperature for these specimens is refrigerate, although frozen also is acceptable.

New Specimen Requirements

(in addition to existing cerebrospinal fluid specimen requirements)

Plasma

Draw blood in a lavender-top (EDTA) tube(s) or yellow-top (ACD) tube(s) (**THROMBIN-ACTIVATED TUBE IS NOT ACCEPTABLE**). Spin down promptly and send 1.0 mL of plasma refrigerate in a screw-capped, plastic vial.

NOTE: 1. Please complete a "Microbiology Request Form" (Supply T244) or a "MayoConnect Additional Test Information Form" (Supply T357) and forward it with the specimen.

2. Label specimen appropriately (plasma).

Urine, Random Collection

Send 1.0 mL of urine refrigerate in a plastic, 13 mL urine tube. Maintain sterility and forward promptly.

NOTE: 1. Please complete a "Microbiology Request Form" (Supply T244) or a "MayoConnect Additional Test Information Form" (Supply T357) and forward it with the specimen.

2. Label specimen appropriately (urine).

Serum Folate Specimen Update

Please note that the correct specimen requirement for #9198 Folate, Serum is a fasting specimen. Nonfasting specimens result in falsely elevated results.

Hepatitis C Virus Quantitation by bDNA Reporting Change

In the past, #81130 Hepatitis C Virus (HCV) Quantitation by bDNA, Serum reported results in both copies/mL and International Units/mL. This assay will now report only in International Units/mL. There will be a conversion factor provided in the report for anyone who wishes to convert from International Units/mL to copies/mL.

Thyroglobulin Assay Interference Alert

As part of our continuous improvement program, we recently identified a higher than acceptable rate of heterophile antibody interference for #9039 [Thyroglobulin, Serum](#). These interferences resulted in falsely elevated serum thyroglobulin measurements in a very small percentage of specimens, approximately 2%. Consequently, Mayo has included additional blocking steps in our analytical procedure to resolve the problem. However, these additional steps may result in an increase in turnaround time of up to 24 hours.

All thyroglobulin results reported after December 17, 2002, can be deemed reliable. Some earlier results may not be accurate and physicians should take extra care when interpreting those results. If the results are not consistent with the clinical picture or other laboratory/imaging studies, retesting should be considered before initiating therapeutic intervention or further diagnostic workup. Specimens are stored for 3 months. If your patient's specimen was tested before the new steps were implemented, but within the last 3 months, the specimen may be available for retesting. Please contact MLI at 800-533-1710 with any questions.

Ask



US

Q: In MML's HIV genotyping test ([#82951 Human Immunodeficiency Virus Type 1\(HIV-1\) Genotyping, Plasma](#)), how are HIV mutation/resistance associations determined? How frequently are they updated?

A: Bayer (previously Visible Genetics, Inc), the company that manufactures this kit, has convened an ongoing consensus panel, comprised of leading experts in the field of HIV resistance, to classify resistance associations. Their process of rules derivation occurs by consensus between all panel members. Visible Genetics is not involved in the decision making during this process. Relevant data presented at a recognized scientific conference or published in a peer-reviewed journal is considered by the panel during this process. In a small number of cases, reliable unpublished data known to panel members may be considered. Rules for determining mutation/resistance associations are based upon one of the following:

- Two or more large, independent virological response studies and supporting in vitro data. In those situations where phenotypic data does not agree with virological response data, virological response data provides the basis for this rule.
- In vitro data (includes phenotypic data and/or in vitro demonstration of mutation selection) and preliminary virological response data
- In vitro data (includes phenotypic data and/or in vitro demonstration of mutation selection). No virological response data is available at the time this rule was devised.
- Extrapolation of data by the consensus panel. Data is extrapolated only in those cases where there is no in vitro or in vivo data regarding one or more mutations or pattern of mutations in a rule. Extrapolation is based upon indirect evidence of resistance effects provided by data from similar antiretroviral agents and/or mutation patterns. Rules based on extrapolated data are included only when the particular antiretroviral for which insufficient data is available is part of routine clinical practice.

The guidelines rules are revised regularly allowing for the incorporation of new data once it becomes available.

2003 Meeting Calendar

Interactive Satellite Programs . . .

Allergic Rhinitis and Its Impact on Asthma

February 11, 2003

Presenter: Gerald Volcheck, MD

Moderator: Robert Kisabeth, MD

New Challenges in Allergy Management: The Role of Primary Care and In Vitro Allergy Testing

March 4, 2003

Presenter: Henry Homburger, MD

Moderator: Robert Kisabeth, MD

Systemic Lupus Erythematosus Diagnosis and Treatment

April 10, 2003

Presenters: Shreyasee Amin, MD & Kevin Moder, MD

Moderator: Steven Ytterberg, MD

The Many Faces of Juvenile Rheumatic Disease

May 13, 2003

Presenters: Thomas Mason, MD & Ann Reed, MD

Moderator: Steven Ytterberg, MD

June 3, 2003

Topic to be determined

Seronegative Spondyloarthropathies: Review and Recent Advances

September 16, 2003

Presenters: Nisha Manek, MD & Clement Michet, Jr, MD

Moderator: Steven Ytterberg, MD

Pharmacogenetics and Pharmacogenomics of Antidepressants

October 7, 2003

Presenters: John Black, MD

David Mrazek, MD

Elliott Richelson, MD

Moderator: Robert Kisabeth, MD

Pharmacogenomics of Warfarin Therapy

November 11, 2003

Presenter: John Height, MD

Moderator: Robert Kisabeth, MD

Cardiac Markers

December 2, 2003

Presenter: Allan Jaffee, MD

Moderator: Robert Kisabeth, MD

Upcoming Education Conferences . . .

Practical Spirometry

February 26-27, 2003

Mayo Clinic, Siebens Building

Rochester, Minnesota

April 29-30, 2003

Radisson Hotel

Rochester, Minnesota

Quality Issues in Phlebotomy

April 10-11, 2003

Mayo Clinic, Siebens Building

Rochester, MN

Integration Through Community Laboratory Insourcing: Implementing a Successful Laboratory Program

March 19-21, 2003

Chateau Sonesta

New Orleans, Louisiana

October 8-10, 2003

Providence Marriott Hotel

Providence, Rhode Island

International Surgical Pathology Symposium

May 6-9, 2003

Four Seasons Hotel

Dublin, Ireland

Practical Surgical Pathology

September 11-13 2003

Mayo Clinic, Siebens Building

Rochester, Minnesota



For a complete listing of all the courses offered throughout the year, contact the Mayo Reference Services Education Office at 1-800-533-1710 or 507-284-8742.

Abstracts of Interest

Presenting Syndromes of Human Immunodeficiency Virus

Robert Orenstein, DO

Over the past 2 decades, numerous changes have occurred in the demographics and clinical course of the human immunodeficiency virus (HIV) infection. Since the initial reports of mucosal candidiasis, severe weight loss, and *Pneumocystis carinii* pneumonia as presenting manifestations of the late stages of immunodeficiency, clinicians have recognized a wide spectrum of manifestations associated with HIV disease. The original reports of severe immunodeficiency were simply the "tip of the iceberg." Advances in antiretroviral therapy and prevention of opportunistic infections have made early diagnosis important. Recognition of cases in earlier stages facilitates opportunities to prevent transmission throughout the population, especially in high-risk groups and pregnant women. Recent evidence suggests that antiviral therapy for primary HIV infection may beneficially alter the course and long-term outcome in persons infected with HIV. This article reviews common presenting syndromes of HIV to aid clinicians in establishing an earlier diagnosis.

Mayo Clinic Proceedings 2002;77:1097-1102

Communiqué

www.mayo.edu/mml/communique.html

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