DIAGNOSTIC, MONITORING, AND RESISTANCE LABORATORY TESTS FOR HIV

What's New — June 2010 Update

Significant revisions include the following:
• Information regarding diagnostic HIV tests has been expanded and updated (see Section II. *Diagnostic Tests*); such tests include:
  o Antibody screening assays
  o Home access tests
  o Rapid tests
  o Western blot for screening oral fluid and urine
• Table 2 has been updated with the latest information regarding available rapid HIV tests
• A table has been added that lists criteria for determining patients who are at risk for HIV-2 infection and should therefore receive combined HIV-1/HIV-2 screening (see Table 3)
• A section on co-receptor tropism detection has been added (see Section III. C. 3. *Co-Receptor Tropism Assay*)
• The committee now recommends performing HLA-B*5701 testing before initiating abacavir-based therapy (see Section III. D. *Human Leukocyte Antigen Testing*)

Key to Abbreviated Terms	
bDNA	Branched chain DNA
CDC	Centers for Disease Control and Prevention
CIA	Chemiluminescent immunoassay
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
HLA	Human leukocyte antigen
NAT	Nucleic acid test
OMT	Oral mucosal transudate
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
RT	Reverse transcriptase
WB	Western blot

I. INTRODUCTION

Technologic advances in HIV laboratory testing continue to aid in screening, diagnosis, and patient management. Effective methods for detection of HIV-related laboratory markers are critical for both accurate HIV screening and for monitoring patients for disease progression and viral resistance to therapy. This chapter provides an overview of currently available HIV laboratory screening methods, viral load assays, and HIV resistance tests. Many new technologies that will further enhance testing methods are in various stages of development.

Patients presenting for testing within 36 hours of exposure to HIV should be assessed for post-exposure prophylaxis (see Post-Exposure Prophylaxis Guidelines). Expert advice may be obtained from the National Clinicians' Consultation Center PEP Line at 1-888-HIV-4911 (1-888-448-4911).

II. DIAGNOSTIC TESTS

RECOMMENDATIONS:

Diagnostic HIV laboratory tests must be performed in full compliance with the New York State HIV Confidentiality Law.

HIV nucleic acid testing (NAT) to detect HIV RNA or DNA, is recommended for establishing the diagnosis of infection in infants born to HIV-1-infected mothers. (AI) See *Diagnosis of Pediatric HIV Infection in HIV-Exposed Infants* for more guidance on infant testing.

Clinicians should use an HIV antibody test with confirmation by Western blot or indirect immunofluorescence assay to establish diagnosis of chronic HIV infection. HIV antibody screening tests include enzyme immunoassays (ELISA/EIA), chemiluminescent immunoassays (CIAs), and rapid tests. (AII)

Patients who test negative for HIV antibody at baseline should receive a follow-up HIV antibody test at 3 months. For individuals who test negative at 3 months but continue to engage in high-risk behavior, clinicians should discuss goal-oriented harm-reduction strategies, including referral for risk-reduction counseling services. Repeat testing at least every 3 months should be offered as long as high-risk behavior continues. (AIII)

Clinicians should evaluate the following populations for acute HIV infection, particularly when they present with a febrile, "flu"-, or "mono"-like illness that is not otherwise explained (see *Diagnosis and Management of Acute HIV Infection*)**:**

- **Those who present for HIV testing** (AIII)
- **Those who report a recent sexual or parenteral exposure with a known HIV-infected partner or a partner of unknown HIV serostatus in the past 2 to 6 weeks** (AII)
- **Men who report having unsafe sexual practices with other men** (AII)
- **Those who report needle-sharing** (AII)
- **Those who present with a newly diagnosed sexually transmitted infection** (AII)
- **Those who present with aseptic meningitis** (AII)
- **Pregnant or breast-feeding patients** (AIII)

A plasma HIV RNA assay should be used in conjunction with an HIV-1 antibody test to diagnose acute HIV infection. Confirmatory HIV antibody testing should be performed 3 to 6 weeks after diagnosis by HIV RNA testing. (AII)

This section discusses the assays available for the diagnosis of HIV infection and how assays are combined to maximize sensitivity and specificity. Categories of available tests include those that detect antibodies against HIV and tests that directly identify circulating viral antigens or RNA/DNA.

HIV immunoassay technology has progressed to include antigens for the enhanced detection of viral variants, such as HIV-1 group O virus and HIV-2. Widespread use of combination HIV-1/HIV-2 enzyme immunoassays (EIA or ELISA), chemiluminescent immunoassays (CIAs), and rapid tests has enhanced the detection of HIV types 1 and 2. In the current generation of HIV-1/HIV-2 immunoassays, the HIV-1 group variants (e.g., M, N, O) will produce an HIV-1-positive result. However, these serologic assays cannot generally provide further discrimination of the exact HIV-1 group (M, N, O) or genetic subtype (A/E, B, C, D, etc.). As a consequence, the need for validated methods that confirm infection with atypical HIV strains has increased. The TaqScreen MPX from Roche is a nucleic acid test (NAT) that has been approved by the Food and Drug Administration (FDA) to simultaneously screen for HIV-1 group O and HIV-2. However, the current application of the test is for blood and tissue bank screening of samples from blood and organ donors. The TaqScreen MPX is not approved for clinical monitoring. Regardless of the diagnostic HIV test used, however, all must be performed in full compliance with the New York State HIV Confidentiality Law.

When acute HIV infection is suspected, a plasma HIV RNA assay should be used, followed by confirmatory antibody testing 3 to 6 weeks later. Most HIV RNA tests will detect acute HIV infection 7 to 14 days after exposure to HIV. A qualitative HIV RNA test is the approved method for such a diagnosis; however, in most cases, a quantitative RNA test may serve this purpose when a qualitative RNA test is not available in a given setting. For further guidance in management of acute HIV infection, see *Diagnosis and Management of Acute HIV Infection*.

A. Antibody Tests

Detection of HIV antibodies is the most common method for the diagnosis of HIV infection in adults and children >18 months old. These antibodies are usually detectable within 3 to 6 weeks after infection, and almost all individuals seroconvert by 12 weeks. However, in rare cases, antibodies may not be detected for months.

Key Point:
Antibody test results that are initially negative should be followed up with HIV antibody testing at 3 months to identify HIV infection in individuals with recent exposures who may not yet have seroconverted at the time of initial presentation.

Serologic testing is currently performed with a highly sensitive screening assay (i.e., ELISA/EIA, CIA, or rapid test), and preliminary positive specimens are followed up with a highly specific confirmatory assay (i.e., Western blot). Antibody testing can also be performed on oral fluid and urine samples when the specific test has been validated for these specimen types. The terms “reactive,” “nonreactive,” and “indeterminate” are used to describe the results of the screening and confirmatory assays. The terms “positive,” “negative,” and “inconclusive” are used to describe the final interpretation of results for a specimen. There are a number of reasons for false-positive, false-negative, and indeterminate/inconclusive results (see Table 1).

TABLE 1 **REASONS FOR FALSE-POSITIVE, FALSE-NEGATIVE, AND INDETERMINATE RESULTS IN ASSAYS FOR THE DETECTION OF ANTIBODIES AGAINST HIV**	
Reasons for False-Positive HIV Screening Test Results	• Increased sensitivity of assays, leading to reduced specificity • Technical errors • Presence of HIV antibodies in recipients of HIV-1 trial vaccines Other rare possibilities: • Hypergammaglobulinemia/antibodies reactive to cellular components • Influenza vaccination may cause cross-reactivity with HIV antibody assays. The time course for such cross-reactivity remains uncertain.
Reasons for False-Negative HIV Screening Test Results	• Testing individuals during the window period (the incubation period between exposure and seroconversion) • Technical errors • HIV-2 (for tests designed to detect only HIV-1) Other rare possibilities: • Delayed antibody synthesis in infants and persons receiving post-exposure prophylaxis or with concurrent acute hepatitis C infection • Diminished immune response in individuals receiving intensive or long-term immunosuppressive therapy • Congenital or drug-induced hypogammaglobulinemia or agammaglobulinemia • Insufficient host antibody response (i.e., advanced HIV disease) • Unavailability of antibodies due to the formation of antigen-antibody complexes • Reduced sensitivity assays
Reasons for Indeterminate* Western Blot Results	**Probable True Positive** *(HIV Infection)* • Seroconverting • HIV-2 infection • Technical errors

	Probable True Negative *(No HIV Infection)*
	• Recipients of HIV-1 trial vaccine
	• Antibodies reactive to cellular components, as in
	   o Multiparous women
	   o Polytransfused patients
	   o Patients receiving chronic hemodialysis
	   o Patients with autoimmune disease
	• Recipients of influenza and hepatitis B virus vaccines
	• Persons with non-HIV acute viral infections
	• Congenital bleeding disorders
	• Alcoholic hepatitis and other chronic liver diseases
	• Hematologic malignancies, lymphomas
	• Positive rapid plasma reagin test
	• Technical errors

* An *indeterminate* WB result is one that cannot be classified as positive or negative based on the test results. The terms *inconclusive* or *nondiagnostic* are synonymous. At this time, there is no clear explanation for this result. The majority of people without risk factors who have indeterminate results (e.g., Red Cross blood donors) do not have HIV. However, HIV still requires consideration when an indeterminate result is obtained, and the test should be repeated in 2 to 3 months to exclude seroconversion.

1. HIV-1 Antibody Screening Assays

Serologic antibody screening tests are relatively easy to perform, allow for large numbers of individuals to be screened, are available from a number of commercial companies, and are designed to be highly sensitive, thus helping ensure the identification of all chronically HIV-infected individuals who are tested. All of the currently available rapid tests and most commercial and public health screening programs rely on ELISA-based technology.

The FDA has approved assays that test body fluids other than blood (primarily oral fluids) for the detection of antibodies to HIV-1 and HIV-2. Three advantages to these assays are as follows:

1) Collection of the sample is noninvasive
2) The absence of needles increases the safety for the personnel collecting the sample
3) Disposal of potentially infectious waste is minimized

The sensitivity and specificity of each test is included in the package insert; however, data included in the package insert are derived from clinical trials and may vary greatly from experience with the actual clinical use of the test. The sensitivity and specificity of a particular test will depend on the prevalence of HIV in the population, the biological fluid examined (i.e., whole blood, plasma, serum, oral fluid, urine, etc.), and the conditions under which the test is performed. Over the years, advances in technology have improved the sensitivity and specificity of these tests; however, the general test methodologies have remained the same (see Appendix B). ELISAs can be configured to detect antibodies or viral antigens through the use of antigens and antibodies as detection reagents, respectively. The fourth generation ELISAs, which had previously been used only in screening blood bank donations, combine both antigen and antibody detection methodologies. The Architect HIV Combo assay (Abbott) has been approved by the FDA for clinical use; this fourth generation ELISA combines detection of HIV p24 antigen and HIV-1/HIV-2 antibodies. Because the HIV p24 antigens produced by the virus may

be detectable before an individual produces antibodies to HIV, the time to HIV detection would decrease with this assay. In one study, the Architect HIV Combo assay detected approximately 88% of acutely infected individuals who had been missed by a third generation ELISA test.[1]

The available screening assays (i.e., rapid tests, the third generation or earlier ELISAs, and CIAs) use recombinant antigens and have markedly shortened the time period between infection and the detection of diagnostic antibodies; the detection of antibodies to HIV-1 infection now averages 21 days following exposure, approximately 1 week longer than detection by NAT.

Samples that are reactive by ELISA are further tested by a more specific assay to confirm infection. Samples nonreactive by ELISA are reported as negative for HIV. No further testing is required for samples reported as negative.

In addition to blood, HIV immunoassay can be performed on oral fluid and urine. One FDA-licensed device (OraSure) is available for the collection of oral mucosal transudate (OMT). Antibodies are readily detectable in OMT but are present at concentrations 800- to 1000-fold lower than those found in serum. OMT is tested using the same algorithm as serum (ELISA followed by a WB when indicated). Both the sensitivity and the specificity of the OMT ELISA have been demonstrated to be slightly lower than currently licensed diagnostic serum tests. Higher rates of false-negative results from the OMT ELISA have been found[2]; however, as of July 2008, these results remain unexplained (see *Dear Colleague Letter: False-Positive Results with Use of Oral Fluid Rapid Test*).

Antibodies to HIV-1 have also been detected in urine. One FDA-licensed HIV-1 ELISA is available for the detection of antibodies in urine (HIV-1 Urine EIA, Calypte Biomedical). As with all antibody screening assays, this method requires confirmation by WB.

Home Access HIV-1 Test System/Dried Blood Spot

The FDA has licensed a home-collection kit that is used to detect antibodies to HIV-1. The individual collects blood from a finger stick and transfers the blood onto filter paper. The sample is then mailed to a facility for analysis by tests that have been approved by the FDA. Pre- and post-test counseling in the case of a negative result consist of a recorded message. For a positive result, a trained HIV counselor will conduct post-test counseling over the telephone. If requested, a counselor is also available in cases of a negative result. Results are available in either 3 or 7 days. Only one HIV home-collection system has been approved by the FDA for sale in the United States. This test, sold as either the “The Home Access HIV-1 Test System” or “The Home Access Express HIV-1 Test System,” is manufactured by Home Access Health Corporation. Many non-FDA-approved kits with questionable reliability are marketed illegally over the Internet and in newspaper and magazine advertisements.

Rapid Test

Rapid tests for HIV are assays that detect antibodies to HIV within minutes. Many of these tests use easily collected fingerstick blood and oral fluid samples and have one-step procedures that are reliable for screening. Some require little training to perform and interpret; a positive result is indicated by the presence of a pink line or circle in the appropriate area. The specific tests that have been approved, the indications for use, and the manner in which they are used are evolving.

Table 2 lists the characteristics of each of the current FDA-approved rapid tests. Additional information is also available on the Food and Drug Administration (FDA) website.

All of the rapid tests detect antibody to HIV and thus will not detect very recent infection with any more accuracy than the standard HIV antibody tests. All have sensitivity and specificity similar to standard HIV antibody tests and similar positive and negative predictive values. They were tested with specimens from patients with potentially interfering substances, including anti-nuclear antibody, C-reactive protein, infectious mononucleosis, and antibodies to HCV, EBV, CMV, HSV, rubella, rheumatoid arthritis, varicella, HAV, HBV, HCV, syphilis, mycoplasma, and streptolysin-O. Samples from patients receiving anticoagulants and from those who had chemical derangements of the blood also had predictive values similar to those from normal samples.

In New York State, a rapid test is defined as an HIV screening test that produces results within 60 minutes or less. Rapid testing is the method of choice when immediate information is necessary to determine the need for prophylaxis, such as in the labor/delivery, newborn, or post-exposure settings, or when the person who is being tested is unlikely to return for a follow-up visit.

When rapid testing is performed, preliminary positive test results should be given to the patient before confirmatory test results are available. Confirmatory WB testing of preliminary positive test results should be completed as soon as possible. Specific protocols and test methods are outlined in Section II. A. 2: HIV-1 Confirmatory Antibody Assays.

Each of the rapid tests is restricted to the body fluid(s) it was designed to analyze (see Table 2). A "waived" test means that the FDA has established that it can be performed by persons with limited training under the auspices of a clinical laboratory (see the FDA's CLIA Certificate of Waiver Fact Sheet for more information), whereas a test without a waiver is characterized as being a nonwaived test of "moderate complexity" and must be performed by only certified personnel at a licensed laboratory (see Table 2). When issuing certificates for nonwaived tests, the New York State Department of Health inspects testing facilities and personnel for compliance with the federally mandated Clinical Laboratory Improvement Amendments (CLIA).

TABLE 2 CHARACTERISTICS OF FDA-APPROVED RAPID HIV TESTS							
Characteristic	FDA-Approved Rapid HIV Tests						
	OraQuick Advance Rapid HIV-1/2	Reveal G3 Rapid HIV-1	Multispot HIV-1/HIV-2	Uni-Gold Recombigen HIV	Clearview HIV 1/2 Stat-Pak	Clearview Complete HIV 1/2	**INSTI HIV-1 Antibody Test**
Manufacturer	OraSure Technologies	MedMira, Inc.	Bio-Rad Laboratories	Trinity Biotech	Inverness Medical	Inverness Medical	bioLytical Laboratories
Detection	HIV-1 and -2	HIV-1	HIV-1 and -2	HIV-1	HIV-1 and -2	HIV-1 and -2	HIV-1
Specimen	Oral fluid, whole blood, plasma	Serum, plasma	Serum, plasma	Whole blood, serum, plasma	Whole blood, serum, plasma	Whole blood, serum, plasma	Whole blood, plasma
Sensitivity[a] (95%)	99.3% (oral fluid); 99.6% (whole blood[b]); 99.6% (plasma)	99.8%	100%	100% (whole blood[b]); 100% (serum, plasma)	99.7% (whole blood[b]); 99.7% (serum, plasma)	99.7% (whole blood[b]); 99.7% (serum, plasma)	99.8% (fingerstick whole blood); 99.9% (venipuncture whole blood); 99.9% (plasma)
Specificity (95%)	99.8% (oral fluid); 100% (whole blood[b]); 99.9% (plasma)	99.1% (serum); 98.6% (plasma)	99.93%	99.7% (whole blood[b]); 99.8% (serum, plasma)	99.9% (whole blood[b]); 99.9% (serum, plasma)	99.9% (whole blood[b]); 99.9% (serum, plasma)	99.5% (fingerstick whole blood); 100% (venipuncture whole blood); 100% (plasma)
CLIA category[c]	Waived: oral fluid and whole blood[b] only	Moderate Complexity: No waiver	Moderate Complexity: No waiver	Waived: whole blood[b] only	Waived: whole blood[b] only	Waived: whole blood[b] only	Moderate Complexity: No waiver

CLIA, Clinical Laboratory Improvement Amendments.
[a] Data shown are for HIV-1 only. For HIV-2 data, see package inserts.
[b] Fingerstick and venipuncture.
[c] Information regarding CLIA waivers of HIV tests is available at www.cdc.gov/hiv/topics/testing/resources/factsheets/roltCLIA.htm. Information about or assistance with completing the CLIA waiver application can be obtained by calling Centers for Medicare & Medicaid Services toll-free at: 1-877-267-2323.

Western Blot for Screening Oral Fluid (OMT) and Urine

A Western blot (WB) that is performed on sample specimens other than blood, such as OMT or urine, should be used only for screening and not confirmatory testing. As indicated above, OMT antibody concentrations are often substantially lower than serum. For HIV-1 antibody detection in urine, one WB test has been approved (Cambridge Biotech HIV-1 Western Blot, Maxim Biomedical). The interpretative criteria for a reactive WB for urine require only the presence of a visible band at the gp160 region. False-positive and false-negative results have been described with this test, and patients should be counseled regarding the reduced sensitivity and specificity of this test. As with all positive screening tests, a serum WB is the mandated follow-up procedure.

2. HIV-1 Confirmatory Antibody Assays

The confirmatory tests for HIV greatly increase specificity of detection when used in conjunction with screening assays. In most cases, they are at least as sensitive as and more specific than screening assays, although not as sensitive in the detection of early seroconversion. Confirmatory tests are more labor intensive, more prone to subjective interpretation, and more expensive than screening assays. The primary role of confirmatory testing is to ensure that individuals who test reactive by screening assays are not incorrectly identified as being infected with HIV.

Confirmatory Western Blot

The most common confirmatory assay for HIV antibody, the Western blot (WB), is the "gold standard" for HIV diagnostic testing (see Appendix C). The virus is disrupted, and the individual proteins are separated by molecular weight via differential migration on a polyacrylamide gel and blotted onto a membrane support. HIV serum antibodies from the patient are allowed to bind to the proteins in the membrane support, and patterns of reactivity can be visibly read. The three major viral bands for HIV are the core protein p24 and the two envelope proteins gp41 and gp120/160. A reactive WB demonstrates antibody to two of the three major bands. A nonreactive WB will have no detectable viral bands. Specimens that are reactive by ELISA and reactive by the confirmatory assay are reported as positive for antibody to HIV-1. Samples that are repeatedly reactive by ELISA but are nonreactive by the confirmatory assay are negative for antibody to HIV-1. A WB in which serum antibodies bind to any other combination of viral bands is considered indeterminate, and a follow-up blood specimen should be obtained 1 month later for repeat HIV antibody testing. Individuals with repeatedly indeterminate results may undergo further testing using NAT, to help resolve infection status. Collection of a new specimen for NAT may be necessary if the initial specimen submitted for serologic testing was not collected and/or handled according to the specifications required for NAT. Because of their potential for yielding false-positive results, these tests should be obtained only in consultation with an expert or other provider who is knowledgeable about testing procedures.

Indirect Immunofluorescence Assay

The indirect immunofluorescence assay (IFA) (see Appendix D) is another confirmatory assay. This test is generally simple to perform but the results are analyzed microscopically and require expertise for interpretation (see Appendix A for description of this procedure).

3. HIV-2 Antibody Screening

RECOMMENDATION:
Clinicians should screen patients who are at risk for HIV-2 infection with a test that detects HIV-2 screening antibodies (see Table 3)**.** (AIII)

Although the prevalence of HIV-2 is low in New York State and elsewhere in the United States,[3,4] individuals who meet the criteria in Table 3 should receive an HIV-1/HIV-2 combination screening test. Many laboratories use a combination ELISA that detects antibodies to both HIV-1 and HIV-2, and FDA-approved rapid tests are available for the detection of HIV-1 and HIV-2 (see Table 2),

TABLE 3 PATIENTS WHO SHOULD RECEIVE COMBINED HIV-1/HIV-2 SCREENING
Patients should receive combined HIV-1/HIV-2 screening when they have: • Had sexual or needle-sharing contact or other drug-using activities in which there was potential for exposure to blood and body fluids with persons who are infected with HIV-2 • Had sexual or needle-sharing contact or other drug-using activities in which there was potential for exposure to blood and body fluids of persons from HIV-2-endemic regions (Angola, Mozambique, and West African countries, including Cape Verde, Ivory Coast, Gambia, Guinea-Bissau, Mali, Mauritania, Nigeria, Sierra Leone, Benin, Burkina Faso, Ghana, Guinea, Liberia, Niger, Sao Tome, Senegal, and Togo) • Received blood products or participated in vaccine trials in an HIV-2-endemic region • Was born to a mother with HIV-2 infection • Has opportunistic infections or other clinical symptoms of HIV infection but tested negative for HIV-1 • Received multiple HIV-1 indeterminate antibody test results (in which WB indeterminate band patterns are suggestive of HIV-2 infection) • A confirmed diagnosis of HIV-1 but an undetectable viral load that is incompatible with the clinical history, suggesting HIV-2 co-infection

When an HIV-1/HIV-2 combination screening test yields a reactive result and an HIV-1 WB yields an indeterminate or nonreactive result, additional testing with an HIV-2 ELISA is required. Alternatively, a Multispot HIV 1/2 rapid test may be used to differentiate reactivity to HIV-1 and HIV-2 antibodies. Any specimen that is specifically reactive for HIV-2 antibodies requires an HIV-2 confirmatory test. Only a limited number of laboratories are capable of performing HIV-2 confirmatory testing, such as NYCDOHMH or NYSDOH laboratories, because there are not any currently available assays licensed by the FDA. However, a test that is labeled for only research use or a laboratory-developed HIV-2 WB or qualitative NAT may be used if the performance characteristics of the test have been fully validated and approved by the laboratory for clinical use.

B. Viral Identification Assays

The implementation of HIV molecular methods to characterize and manage HIV infection has changed how HIV medicine is practiced. Polymerase chain reaction (PCR), nucleic acid sequence-based amplification (NASBA), branched DNA (bDNA), and transcription-mediated amplification (TMA) methods offer increased sensitivity to:

- Identify early infection
- Determine HIV types, groups, and genetic subtypes

1. DNA Polymerase Chain Reaction (DNA-PCR)

RECOMMENDATIONS:

HIV-1 DNA PCR should be used only for the detection of infection in infants born to mothers infected with HIV-1. (AIII)

All initial positive DNA PCRs should be confirmed with a second PCR test on a separate specimen. (AII)

PCR is currently the best-known assay for the amplification of nucleic acid. Although the HIV-1 DNA PCR assay has been used as an investigational tool for more than a decade, it is not licensed in any format by the FDA. This qualitative procedure is very sensitive and can detect between 1 and 10 copies of HIV-1 proviral DNA per sample. Because of the extremely high sensitivity of this assay, small amounts of background "noise" in the environment or contamination during laboratory processing may result in amplification of products that can produce false-positive reactions.[5] All initial positive DNA PCR results require confirmation with a second PCR test on a separate specimen. Currently, the only recommended diagnostic use of HIV-1 DNA PCR is for the detection of infection in infants born to mothers infected with HIV-1. When used in this setting, the potential for a false-positive result should be recognized. The NYSDOH strongly recommends that all New York State birth facilities use the pediatric HIV testing services at the Wadsworth Center. The Wadsworth Center uses the APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe), which has been demonstrated to identify HIV infection an average of 4 weeks earlier in non-breastfed infants when compared to a qualitative DNA-PCR. See *Diagnosis of Pediatric HIV Infection in HIV-Exposed Infants*.

2. Plasma HIV RNA Assays

RECOMMANDATION:

A plasma HIV RNA assay should be used in conjunction with an HIV-1 antibody test to diagnose acute or primary HIV infection. (AII)

The natural history of acute HIV infection is such that antibodies may not have formed at the time of onset of symptoms and viremia (2 to 6 weeks after exposure). Antibody testing of these patients will often yield a negative to weakly positive ELISA and negative or "evolving" WB, in which additional bands may begin to appear with sequential tests performed over the course of seroconversion. However, viral load levels are very high during acute infection, usually ranging from 100,000 to over 10 million copies/mL, and are detectable approximately 2 weeks prior to seroconversion. It is therefore important to use both a plasma HIV RNA assay and an antibody

test to establish the diagnosis. Low levels of virus (a commonly cited cutoff is <5000 copies/mL) may be indicative of a false-positive result and should not be considered diagnostic of primary HIV infection. Standard antibody testing should be repeated in 3 to 6 weeks. Methods used to measure plasma HIV RNA include conventional and real-time reverse transcriptase (RT)-PCR, bDNA, and NASBA. The FDA has recently approved the Aptima HIV-1 RNA Qualitative Assay (Gen-Probe) for diagnosis of acute or primary HIV infection. Further description of these tests is given in Section III. B. Viral Load Assays. Plasma HIV RNA levels during seroconversion do not appear significantly different in patients who have acute symptoms versus those who are asymptomatic.[6]

For further guidance in identification and management of acute HIV infection, see *Diagnosis and Management of Acute HIV Infection*.

III. MONITORING TESTS

Once a patient has been diagnosed as being HIV-infected, several tests are required to evaluate and monitor the clinical progression of disease.

A. Lymphocyte Analysis

RECOMMENDATIONS:
Clinicians should measure CD4 cell counts at the time of diagnosis of HIV infection and every 3 to 4 months thereafter (see *Antiretroviral Therapy*: Section VI. A. 2. Lymphocyte Subsets)**.** (BIII)

Treatment decisions should not be made solely on the basis of a single CD4 cell measurement obtained at a single point in time. Treatment decisions should be made only after two successive measurements have been obtained. (AIII)

CD4 cell counts should not be used for diagnosis of HIV infection.

Low CD4 cell counts are seen in a number of disease processes and should therefore not be used for diagnosis of HIV. However, the CD4 cell count is important for determining the staging of HIV disease and the need for prophylaxis against opportunistic pathogens. CD4 cell counts <200 cells/mm^3 (or <14% of total lymphocytes) meet the national surveillance case definition for AIDS. CD4 cell counts continue to be used to assist in decisions regarding initiation or adjustment of ARV therapy. For persons infected with HIV-2 or HIV-1 variants that cannot be accurately quantitated using viral load assays, CD4 count remains the most effective monitoring tool for progression of disease.

Absolute CD4 cell counts are calculated values that may fluctuate widely. The calculation is made by multiplying the total white blood cell count (in thousands) by the percentage of total lymphocytes and then by the percentage of CD4 lymphocytes. Therefore, any change in one of these three parameters will cause the absolute CD4 count to vary. As a result, CD4 percentage is a direct measurement and more reliable. Fluctuations in the absolute CD4 cell count in the setting of a stable CD4 percentage can assure both the patient and the clinician that immunologic stability is present.

Factors influencing lymphocyte subsets include sex, age, race, drugs (zidovudine, cephalosporins, cancer chemotherapy, nicotine, and corticosteroids), anti-lymphocyte antibodies, and splenectomy. Differences in reagents and equipment both within a laboratory and between laboratories may further contribute to variations in CD4 cell counts. Because of this variability, treatment decisions should not be made solely on the basis of a single CD4 cell measurement obtained at a single point in time. Treatment decisions should be made only after two successive measurements have been obtained. For more information, refer to *Antiretroviral Therapy*: Section III. Deciding When to Initiate ARV Therapy.

B. Viral Load Assays

RECOMMENDATIONS:
Clinicians should repeat viral load tests that are inconsistent with the clinical presentation before management decisions are made. (AIII)

Ultrasensitive assays that detect as few as 25 to 75 copies/mL should be used to monitor patients who have viral loads <400 copies/mL. (BIII)

Several different HIV viral load tests have been developed, and five are currently approved for use in the United States:

- Amplicor HIV-1 Monitor test (Roche), also known as the PCR test
- Versant/Quantiplex HIV-1 RNA, or bDNA (Chiron/Siemens)
- NucliSens HIV-1 QT, or NASBA (bioMérieux)
- Abbott RealTime HIV-1
- COBAS AmpliPrep/COBAS TaqMan HIV-1 Test

Viral load assays quantify the amount of HIV-1 RNA circulating in the blood of an infected individual. Total quantification includes cell-free virus, virus in infected cells in all compartments of the body, and integrated provirus. However, the easiest measurement of viral load is that of cell-free virus in an individual's plasma. Because differences exist in the absolute copy number generated by different viral load assays, the same assay should be used to follow an individual's viral load. For a specific assay, the biologic variability of viral load is 2-fold.

Viral load tests are also approved for monitoring the effects of ARV therapy, to track viral suppression, and to detect treatment failure.[*] Successful combination ARV therapy should decrease viral load 1.5 to 2 logs (30-100 fold) within 6 weeks, with the viral load decreasing below the limit of detection within 4 to 6 months.[7]

Standard assays have a lower limit of detection of 400 copies/mL, and ultrasensitive assays may detect viral loads as low as 5 to 50 copies/mL. Cohort studies strongly suggest that patients with viral loads <50 copies/mL have more sustained viral suppression than patients with viral loads between 50 and 400 copies/mL. Ultrasensitive assays are therefore more useful than standard

[*] Older methods of quantitation of viral burden that are not recommended or no longer have FDA clinical approval include the Mediva SUDS test, quantitative viral culture, and the HIV-1 p24 antigen assay.

viral load tests in predicting prolonged viral suppression and are recommended for monitoring patients who are receiving ARV therapy.

Brief viral rebounds, known as "blips," can result in viral load levels of 50 to 500 copies/mL in patients with previously undetectable viral loads (<50 copies/mL). Acute concurrent illness and/or recent vaccination may cause this transient rise; however, studies have suggested that blips represent random biologic and statistical variation or false elevations of viral load resulting from laboratory processing.[8,9] Blips are not often associated with the development of resistance mutations or virologic failure and do not usually require a change in ARV therapy.[9] Re-testing should be performed after 12 weeks before a change in ARV regimen is considered. For more information, refer to *Antiretroviral Therapy*: Section VI. Monitoring of Patients Receiving ARV Therapy.

1. Roche Amplicor HIV-1 Monitor and Roche Amplicor HIV-1 Monitor Ultrasensitive (RT-PCR)

The Roche Amplicor HIV-1 Monitor Version 1.5 (see Appendix E) and Roche Amplicor HIV-1 Monitor Ultrasensitive Version 1.0 (RT-PCR) are approved by the FDA for the quantitation of HIV-1 RNA in plasma and are reported as copies/mL. This procedure is similar to the DNA PCR assay. For patients with good viral suppression, the ultrasensitive test is the preferred method for quantifying plasma HIV-1 RNA. HIV-1 RNA is isolated from the plasma; then a complementary strand of DNA (cDNA) is transcribed from the target RNA using RT. The cDNA is amplified using very specific oligonucleotide primers. Quantification of the RNA is achieved by hybridizing the amplified DNA to specific probes, followed by a colorimetric detection assay (see Appendix A for further description of this procedure).

2. Branched Chain DNA (bDNA)

The Versant HIV-1 RNA 3.0 assay is approved by the FDA for quantitation of HIV-1 RNA in plasma, and results are reported as units/mL. It uses the bDNA technology to measure viral load (see Appendix F). The bDNA assay consists of a series of hybridization procedures followed by an enzyme substrate reaction (see Appendix A for further description). In this assay, HIV-1 present in the patient's blood is disrupted to release the viral RNA. The RNA is captured by a set of capture probes (bound by solid phase), and a set of target probes hybridizes both the viral RNA and the preamplifier probes. The amplifier probe hybridizes to the pre-amplifier probe, forming a branched DNA (bDNA) complex. The bound bDNA is incubated with an enzyme and then with a chemiluminescence substrate.

3. Nucleic Acid Sequence-Based Assay (NASBA)

The NucliSens HIV-1 QT assay (bioMérieux) is a nucleic acid sequence-based assay that has been approved by the FDA for the quantitation of HIV-1 RNA in plasma. Results are reported as units/mL. In this viral load test, the HIV-1 is lysed and HIV-1 RNA is extracted and bound to silica beads (see Appendix G). Nucleic acid amplification then occurs using specific primers derived from the gag region of the genome. The amplified RNA is hybridized to capture probes attached to magnetic beads, and the nucleic acid is detected by measuring electrochemiluminescence. The isolation technique used in this assay allows diverse sample types (plasma, cerebrospinal fluid, lymph tissue, genital secretions, and cells) to be used as the source of viral nucleic acid; however, the FDA-approved assay, NucliSens HIV-1 QT, has only been validated for use with plasma.

4. Other Viral Load Tests

An inexpensive HIV viral load assay has been developed to measure viral RT activity (ExaVir Load Version 2; Cavidi AB, Uppsala, Sweden). The test is performed mostly manually and was designed primarily for resource-limited settings. The assay has a lower limit of detection of 400 copies/mL. It is not approved by the FDA for clinical use in the United States. Another investigational viral load assay is the real-time immuno-polymerase chain reaction (IPCR), which combines ELISA and PCR methods for quantification of HIV-1 p24 antigen detection.[10]

C. Drug Resistance Tests

RECOMMENDATIONS:

Clinicians should perform resistance testing under the following circumstances:

- **At baseline, regardless of whether ARV therapy is being initiated (genotypic testing)** (AIII)
- **In ARV therapy-naïve patients before initiation of ARV therapy (genotypic testing)** (AII)
- **In patients experiencing treatment failure or incomplete viral suppression while receiving ARV therapy (genotypic and/or phenotypic testing)** (AII)

Resistance testing should be performed promptly in cases of virologic failure or incomplete viral suppression. Resistance testing should be performed while patients are still receiving therapy or have been off therapy for no more than 1 year. (AII)

Clinicians should consult with an expert to interpret the results of resistance assays because such results are often complex (the New York State AIDS Institute's Clinical Education Initiative line is available for phone consultation)**.** (AIII)

The replication mechanism of HIV makes it prone to mutations (i.e., changes in its genetic sequence). Most currently available ARV drugs are targeted to inhibit the activity of two specific viral proteins, the protease and RT. Many mutations have been identified in these proteins that alter the ability of one or more ARV drugs to inhibit the viral protein, making the virus resistant to the drug(s). ARV drugs that inhibit fusion and viral entry and integration are now available; however, resistance mutations for these agents have also been identified.

In numerous studies, the emergence of drug resistance mutations has been associated with virologic failure during ARV treatment. Two general methodologies are used to determine drug resistance for HIV: genotyping and phenotyping. The clinical benefits of using resistance tests to guide treatment decisions for patients at various stages of infection and treatment are discussed in *Antiretroviral Therapy*: Section VI. Monitoring of Patients Receiving ARV Therapy. In New York State, third-party reimbursement programs, including Medicaid, the New York State AIDS Drug Assistance Program (ADAP), and private insurers, often limit resistance testing to no more than three tests per year (within 12 months following date of first use), regardless of the type of resistance test that is performed.

New resistance mutations and the emerging clinical significance of these mutations frequently change. Several resources are available for more information on drug resistance and resistance testing. These include:

- www.medscape.com/hiv
- www.aidsinfo.nih.gov
- hivdb.stanford.edu
- www.hiv.lanl.gov/content/hiv-db/mainpage.html

1. Genotyping

A genotypic assay provides an indirect measure of drug resistance because it is based on detection of the mutations known to be associated with resistance. Genotypic testing involves determining the sequence of the genomic region where resistance mutations occur, typically the coding region for the protein inhibited by the drug. This is best achieved by direct sequencing methods. Two direct sequencing-based methods have been approved by the FDA: the TruGene HIV-1 Genotyping assay (Siemens) and the ViroSeq HIV-1 Genotyping System (Celera Diagnostics). In addition, laboratory-developed ("in-house") genotyping assays are available through several commercial laboratories. Advances in genotyping assays continue to evolve, with many assays still limited to research settings. Although commercial assays are still under development, in-house assays may be available for integrase genotyping and for gp41 genotyping for fusion inhibitors.

In genotyping assays, the HIV-1 RNA is isolated from a plasma specimen and reverse transcribed to produce cDNA. Specific regions of the HIV genome are amplified by PCR and sequenced. This sequence is then compared with that of a drug-sensitive (wild-type) strain of HIV, and differences (mutations) present in the specimen sequence are noted. Computer software is generally used to perform this comparison and to predict whether resistance to specific drugs is likely to result from the particular combination of mutations detected in the virus. For most genotypic assays, this prediction is based on a set of rules derived from clinical observations, laboratory studies, and the advice of experts in the field. The actual prediction of resistance may vary from laboratory to laboratory for some combinations of mutations, depending on the interpretation algorithm used to define the rules. Currently available genotypic assays require a minimum viral load in the range of 500 to 2000 copies/mL, depending on the assay, and generally require 2 weeks or less for results.

One method of genotypic testing, the "virtual phenotype type" (VIRCO, vircoTYPE), uses a variation of the standard rules-based method of interpreting genotypic test results. The patient's mutation profile is analyzed using a comprehensive database consisting of correlated genotypic (sequence) and phenotypic (drug susceptibility) data. A patient's genotype is entered into the database, and viruses with similar genotypes to those of the patient's virus are identified. The drug susceptibility results (IC50 and fold change, see Section 2: Phenotyping) of these matching viruses are used to calculate the probable degree of drug susceptibility of the patient's virus. The report provides the mutations detected by sequencing, the predictive phenotype results, and the number of matches on which the prediction was based. A minimum number of matches to the patient's virus must be present in the database in order to obtain an interpretation. The advantages of this type of virtual phenotypic testing are that the results are available more rapidly and the interpretation is similar to that of a conventional phenotypic assay. However, a disadvantage is that the actual viral phenotype may be different from the result because of limitations of the database.

2. Phenotyping
A phenotypic assay provides a direct measure of drug resistance. The currently available phenotypic assays use recombinant DNA methods to measure the ability of a patient's virus to grow in the presence of a drug. Therefore, results from a phenotypic test include the net effect of any and all resistance mutations.

In the phenotypic assay, HIV RNA is isolated from plasma and converted into cDNA, and the relevant region is amplified by PCR. This amplified material is inserted into a recombinant virus system whereby the susceptibility to different drugs can be tested. The result from the phenotypic assay is an IC50 value that defines the concentration of the drug required to reduce growth of the virus by 50%. The IC50 of the patient's virus is compared to the IC50 of a drug-sensitive (wild-type) reference virus, and the fold change is defined. If the IC50 value of a person's virus is greater than that of the reference virus for a particular drug, it indicates that the person's virus has decreased sensitivity to the drug. The relative fold change helps determine whether the drug should still be part of the therapy regimen or whether it should be removed entirely. Two companies, Tibotec-Virco (Antivirogram) and Monogram-Biosciences (PhenoSense), offer phenotypic resistance testing through many clinical laboratories. Phenotypic assays have a minimum viral load requirement of 500 to 1000 copies/mL and generally require 3 to 5 weeks for results. Phenotypic assays are more technically complex, labor-intensive, and expensive than genotypic assays; however, they may provide a more accurate indication of drug susceptibility, particularly when a patient's virus presents a complex mutation profile.

3. Co-Receptor Tropism Assay
Co-receptor tropism analysis determines the type of cellular co-receptor (either CCR5 or CXCR4) that an HIV-infected individual's dominant viral population uses to gain access to host cells. The majority of acutely or recently infected individuals, including perinatally infected children, have a CCR5-utilizing virus. The drugs that target the CCR5 co-receptor, such as maraviroc, will likely be effective in these patients (see *New Antiretroviral Drugs: Maraviroc, Raltegravir, and Etravirine*).

Because the CCR5-tropic virus predominates early in HIV infection, whereas CXCR4-tropic virus is often present in late-stage disease, the CCR5 variant may be preferentially transmitted compared to CXCR4 variants. In chronically HIV-infected individuals, a population of mixed CCR5- and CXCR4-tropic viruses, as well as viruses with both tropisms, is also commonly encountered. Such viral populations are often referred to as dual/mixed or D/M HIV.

Co-receptor tropism testing is currently performed using phenotypic testing. Although phenotypic testing can determine a viral population containing both tropisms, it is not sufficiently sensitive to differentiate between mixed and dual tropism.

The Trofile (Monogram Biosciences) co-receptor tropism assay permits phenotypic identification of CCR5, CXCR4 co-receptor, or dual/mixed-tropic (CXCR4/CCR5-utilizing) HIV-1 before the initiation of co-receptor antagonist ARV therapy. A second assay, the HIV-1 Coreceptor Tropism (Quest Diagnostics), uses molecular heteroduplex tracking method to detect CXCR4-tropic HIV-1. Other genotypic-based assays are under development but are not yet available.[11,12]

Another commercially available recombinant phenotypic assay for assessing HIV chemokine co-receptor tropism is the Phenoscript assay (Eurofins VIRalliance). In this assay, a 900-bp portion containing the patient's V1-V3 envelope virus is amplified and inserted into a HIV-1 vector lacking the corresponding V1-V3 section. The fully complemented HIV-1 is then able to produce virus that can be used to infect cell lines with either CCR5 or CXCR4 on their surfaces with a colorimetric readout. The results are reported in a similar manner as the Trofile (i.e., CCR5-trophic, CXCR4-trophic, or dual/mixed tropic). This assay has not been validated in a clinical trial setting or against the Trofile assay.

Resistance to the new class of CCR5 co-receptor antagonists develops by two unrelated mechanisms. First, the patient's viral population shifts its co-receptor usage (i.e., uses CXCR4 exclusively or uses both CCR5 and CXCR4 receptors to gain entry into the cell). The current assays are not sufficiently sensitive to discriminate between mixed- or dual-tropic populations. The second method by which resistance to a CCR5 receptor antagonist may develop is by the virus mutating and binding to the CCR5 receptor with the drug antagonist still in place. This second method can be discerned by a flattening of the IC90 curves in a phenotypic assay or potentially by genotypic analysis. Analysis by phenotypic assay is the preferred method for this purpose because genotypic data are more complex.

4. Other Drug Resistance Tests

Replicative Capacity

Replicative capacity information is often provided as an adjunct to phenotypic or combination genotypic-phenotypic resistance assays. Currently, the test is configured by inserting patient-derived RT and protease sequences into a modified retroviral vector containing a luciferase indicator gene that allows quantification of viral replication. After normalizing the output of the assay on the basis of the viral inoculum, the ability of the vector to replicate is measured in the absence of an ARV drug. The relative replicative capacity of the virus from the source patient is calculated as the ratio of the luciferase activity from vectors containing patient-derived sequences to the luciferase activity from vectors containing wild-type sequences. A ratio of less than 1 reflects a reduced replicative capacity as compared with that of the wild-type control. The full clinical value of this adjunctive information remains under investigation.

D. Human Leukocyte Antigen Testing

RECOMMENDATION:

Clinicians should perform HLA-B*5701 testing before initiating abacavir-based therapy. (AI)

Individuals with human leukocyte antigen (HLA)-B*5701, HLA-DR7, and HLA-DQ3 have an ostensible genetic predisposition to development of abacavir hypersensitivity. HLA-B*5701 testing is the most thoroughly documented and may be useful for addressing concerns about treatment with abacavir.[13,14] See *Antiretroviral Therapy*. Unlike virus-specific tests (HIV genotype, phenotype, co-receptor tropism assays), HLA genotyping is necessary only once during an individual's lifetime, because it will not change over time.

References

1. Pandori MW, Hackett J Jr, Louie B, et al. Assessment of the ability of a fourth-generation immunoassay for human immunodeficiency virus (HIV) antibody and p24 antigen to detect both acute and recent HIV infections in a high-risk setting. *J Clin Microbiol* 2009;47:2639-2642. [PubMed]

2. Centers for Disease Control and Prevention. False-positive oral fluid rapid HIV tests--New York City, 2005-2008. *MMWR Morb Mortal Wkly Rep* 2008;57:660-665. [PubMed]

3. Kanki PJ, DeCock K. Epidemiology and natural history of HIV-2. *AIDS* 1994;8:S85-S93.

4. Acquired immunodeficiency syndrome (AIDS). Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2, and HTLV-1/HTLV-II. *Wkly Epidemiol Rec* 1990;65:281-283.

5. Louie M, Louie L, Simor AE. The role of DNA amplification technology in the diagnosis of infectious diseases. *CMAJ* 2000;163:301-309. [PubMed]

6. Schacker T, Collier AC, Hughes J, et al. Clinical and epidemiologic features of primary HIV infection. *Ann Intern Med* 1996;125:257-264. [PubMed]

7. DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. December 2009. Available at: www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf

8. Nettles RE, Kieffer TL, Kwon P, et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. *JAMA* 2005;293:817-829. [PubMed]

9. Lee PK, Kieffer TL, Siliciano RF, et al. HIV-1 viral load blips are of limited clinical significance. *J Antimicrob Chemother* 2006;57:803-805. [PubMed]

10. Barletta JM, Edelman DC, Constantine NT. Lowering the detection limits of HIV-1 viral load using real-time immuno-PCR for HIV-1 p24 antigen. *Am J Clin Pathol* 2004;122:20-27. [PubMed]

11. Vandekerckhove L, Verhofstede C, Vandenbroucke I, et al. Direct comparison with 454 pyrosequencing validates V3-loop based genotyping in patients eligible for Maraviroc initiation. ICAAC, September 15, 2009, San Francisco, CA. Abstract.

12. McGovern RA, Dong W, Mo T, et al. Optimization of clinically relevant cutpoints for the determination of HIV co-receptor usage to predict maraviroc responses in treatment-experienced (TE) patients using population V3 genotyping. EACS, November 13, 2009. Cologne, Germany. Abstract.

13. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse transcriptase inhibitor abacavir. *Lancet* 2002;359:727-732. [PubMed]

14. Hetherington S, Hughes AR, Mosteller M, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet* 2002;359:1121-1122. [PubMed]

APPENDIX A

ASSAY PROCEDURES

I. Enzyme-Linked Immunosorbent Assay (ELISA)

In the first step of this procedure, patient serum or plasma is added to the viral antigens attached to a solid support and allowed to react. If present, specific antibodies to the virus will bind to such antigens. A series of subsequent steps involving incubation with an anti-human immunoglobulin attached to an enzyme followed by incubation with a substrate will result in the production of a signal (often a color change). This color change is measured by a spectrophotometer, and its relationship to the positive and negative controls of the test serve to quantitate the extent of antibody-antigen complex formation. Between each step of the assay, unbound antibodies or reagents are washed away from the reaction site prior to the initiation of the next step.

II. Western Blot (WB)

Serum is allowed to react with the viral proteins on the membrane, and then an enzyme-substrate reaction is performed to visualize immunoreactive bands. Nine HIV-specific antigen bands are monitored: gp160, gp120, p66, p55, p51, gp41, p31, p24, and p17. Of these bands, gp160, gp120, gp41, and p24 are the major bands of diagnostic importance.

III. Immunofluorescence Assay (IFA)

Patient serum is reacted with HIV-infected cells and with control uninfected cells in wells on microscope slides. A fluorochrome is used as the indicator system (instead of the enzyme substrate system used for both the WB and the screening ELISA). The use of control wells allows for the detection of nonspecific reactions.

IV. Viral Culture

Incubation of the patient's peripheral blood mononuclear cells (PBMCs) with mitogen-stimulated PBMCs obtained from an HIV-negative donor is allowed to occur. A sample of the growth medium from these co-cultures is routinely removed and tested for RT activity or for the presence of p24 viral antigen. Fresh medium and freshly stimulated donor PBMCs are added as needed to maintain the cultures for up to 1 month. A positive culture is demonstrated by the detection of RT activity or p24 antigen in the supernatant fluid.

V. DNA Polymerase Chain Reaction (PCR)

The individual's PBMCs are harvested, cellular DNA is extracted, and target DNA is amplified using a very specific set of oligonucleotide primers. The reaction progresses through 30 to 35 cycles of denaturation, annealing, and synthesis of new DNA, ending with billions of copies of the target DNA.

VI. Lymphocyte Analysis

Whole blood is incubated with lymphocyte-specific monoclonal antibodies. A lysing reagent is added to remove red blood cells. The cell analysis is then performed, using a flow cytometer, an instrument composed of three interacting elements: a computer, a laser, and a fluidics system. Fluorescent labels attached to the monoclonal antibodies have specific light absorption and emission wavelengths. Each wavelength is characterized by a specific color of light emission. This information is captured, collected, and collated in a computer. Cells, one at a time, pass in front of the laser, producing light scatter and fluorescent light emission. Cell populations are identified and characterized by their size, granularity, and intensity of fluorescence.

VII. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

This test uses RNA from an individual's plasma. An oligonucleotide primer specific to HIV-1 is used to synthesize a DNA copy (cDNA) of the HIV RNA. This cDNA is then amplified by PCR for either quantitative or qualitative measurement. Although this procedure is quite specific, its sensitivity may be reduced when the virus strain causing the infection is an HIV-1 group M non-subtype B variant. Generally, HIV-1 group O and HIV-2 will not be amplified or will have a greatly reduced amplification. As with all methods that involve oligonucleotide binding, the genetic differences in HIV-2 strains in comparison with HIV-1 may impair oligonucleotide binding in RT-PCR. For RT-PCR, this can reduce the efficiency of either the RT or PCR step, reducing or preventing amplification even if the amount of HIV-2 virus is high. Similar problems may be observed with HIV-1 group O or even some group M HIV-1 strains, depending on the assay design.

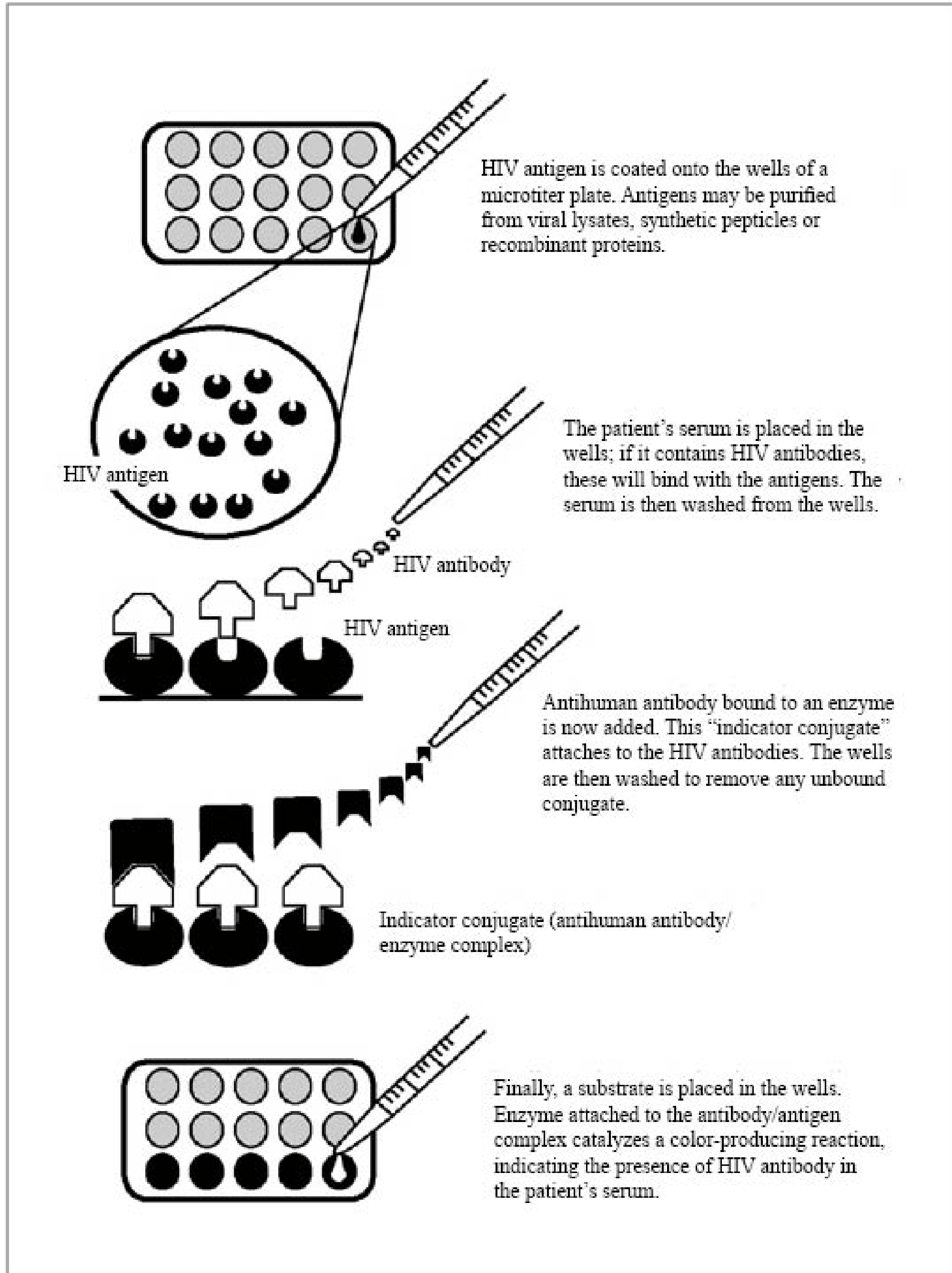
An additional factor for reduced amplification of HIV-2 is that viral loads are generally lower for HIV-2 (i.e., the viral load set point), as determined by assays optimized for HIV-2 quantification, and may possibly explain the lower pathogenicity and reduced amplification of HIV-2 compared to HIV-1. For specific quantification of HIV-2 viral load, an assay designed specifically for that purpose is preferred. However, such assays are limited to those developed in-house by laboratories because no FDA-approved HIV-2-specific viral load assay is presently available.

VIII. DNA Sequencing

In general, a specific region of the HIV RNA is amplified by RT-PCR. Copies of the RT-PCR product are synthesized using a specific oligonucleotide primer and reaction terminators that ultimately result in products that stop at every position along the region of DNA. A fluorescent label is incorporated into these products during the sequencing reaction. The products in the reaction mix are then separated and sorted by polyacrylamide gel electrophoresis and analyzed to determine the order in which the nucleotides occur in the region of interest.

APPENDIX B

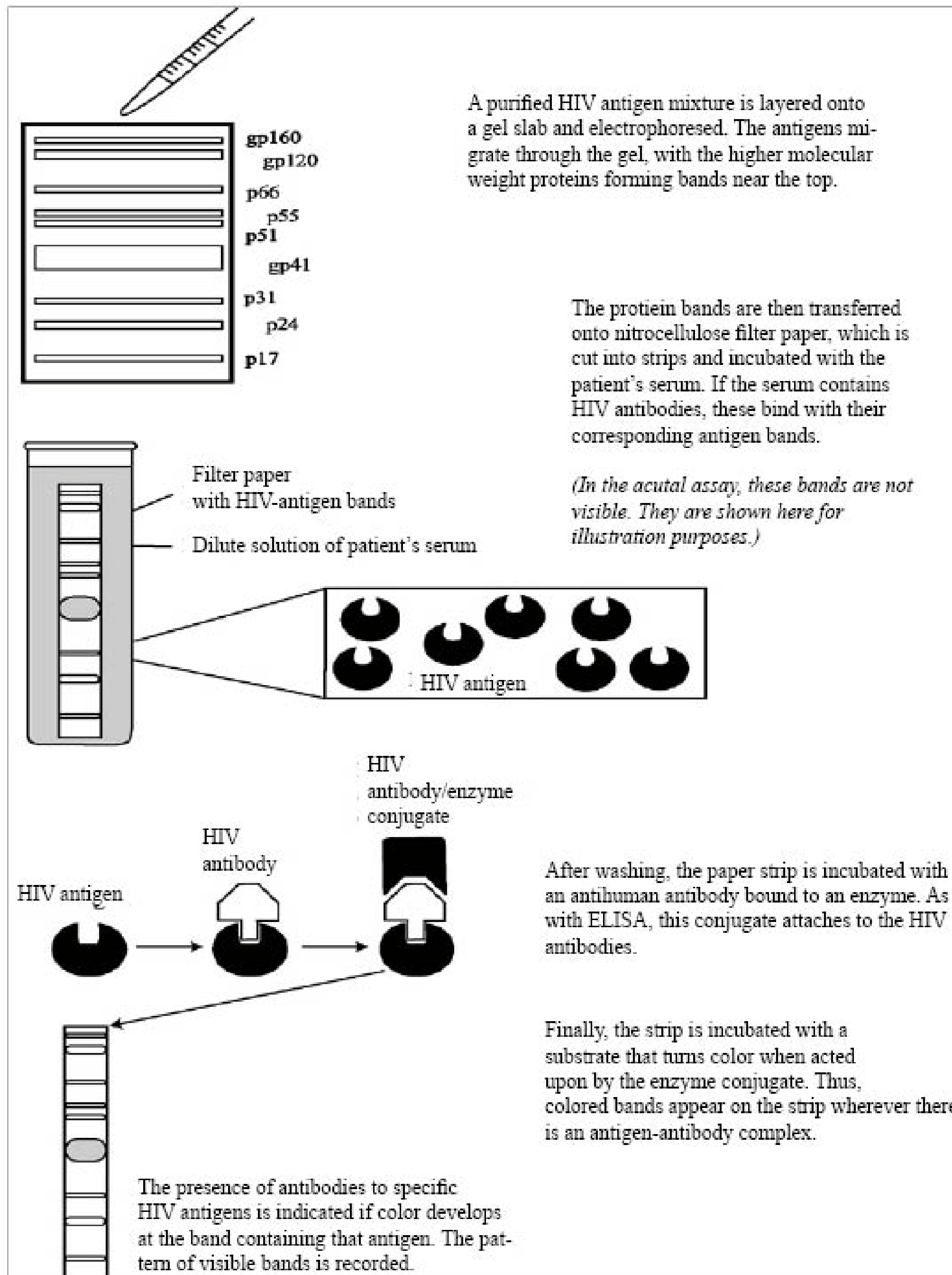
Enzyme-linked Immunosorbent Assay



Provided courtesy of Sara T. Beatrice, PhD.

Appendix C

Western Blot



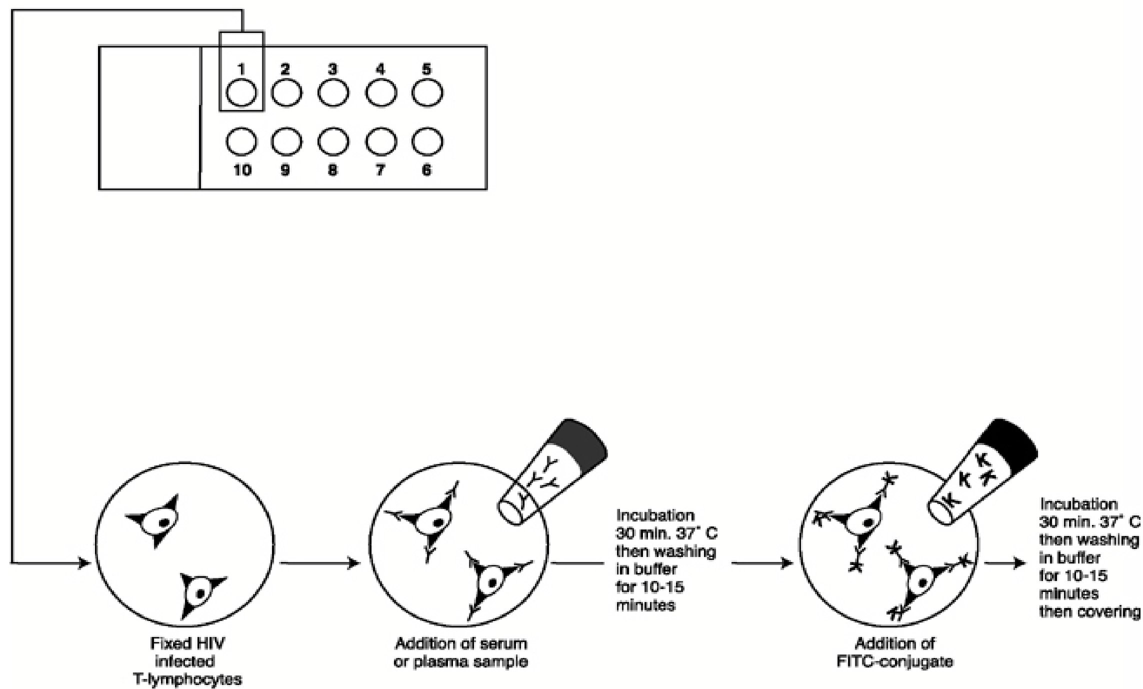
Provided courtesy Sara T. Beatrice, PhD.

APPENDIX D

Immunofluorescence Assay (IFA)

PRINCIPLE

Indirect immunofluorescence assay for the detection of antibodies to HIV in serum or plasma.



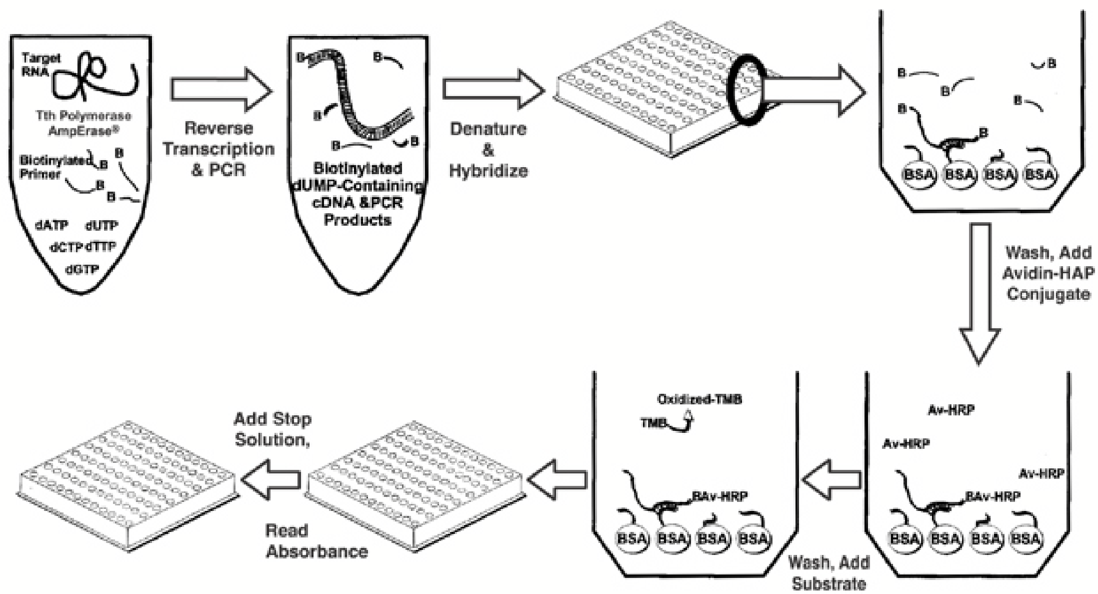
EVALUATION UNDER THE FLUORESCENCE MICROSCOPE

HIV-infected T-lymphocytes originating from a patient suffering from lymphatic leukemia are fixed on a slide and covered with the blood sample to be tested. If antibodies are present these are bound to the virus antigens and remain adherent to the infected cells even after washing. For detection of the bound antibodies, FITC-conjugated anti-human globulin is added and rinsed off after incubation. If the sample contains antibodies against HIV, cells show a bright membrane fluorescence under the microscope.

Provided courtesy Sara T. Beatrice, PhD.

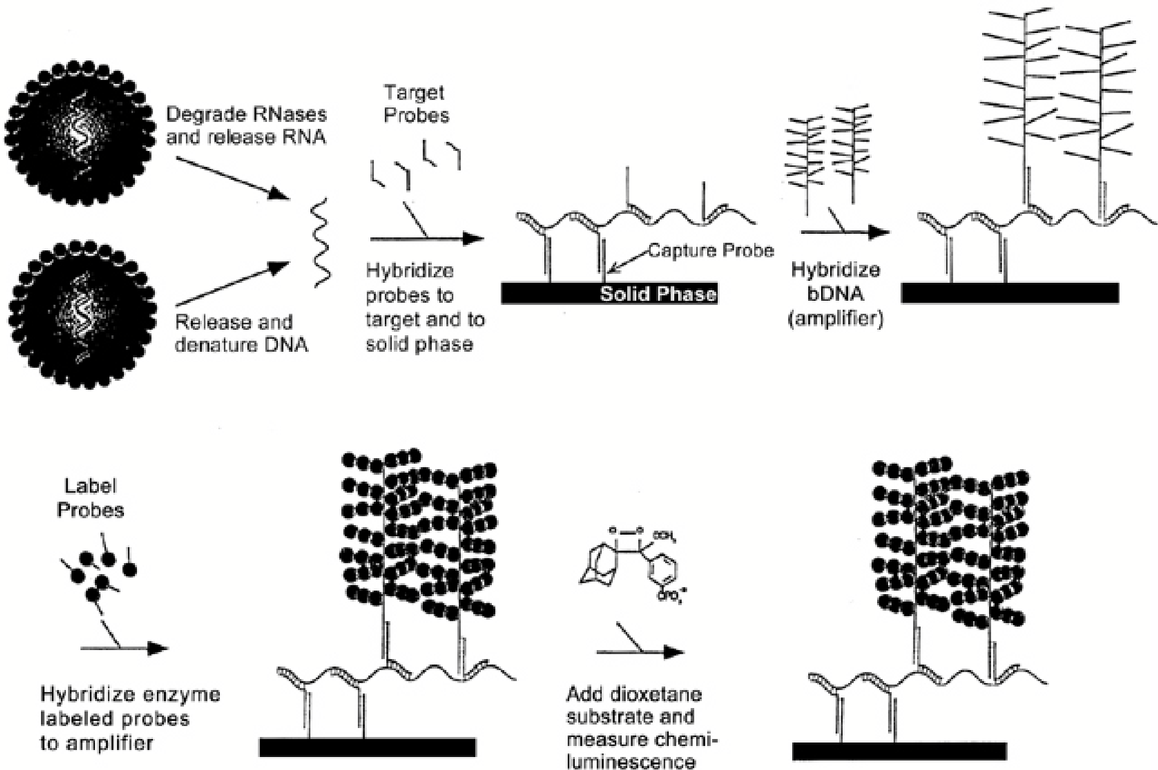
APPENDIX E

Amplicor HIV-1 Monitor Test Format



APPENDIX F

Versant bDNA Signal Amplification Assay



APPENDIX G

Nucleic Acid Amplification System