TABLE 226-8 CHARACTERISTICS OF TESTS FOR DIRECT DETECTION OF HIV

Test	Technique	Sensitivity[a]	Cost/Test[b]
Immune complex–dissociated p24 antigen capture assay	Measurement of levels of HIV-1 core protein in an EIA-based format following dissociation of antigen-antibody complexes by weak acid treatment	Positive in 50% of patients; detects down to 15 pg/mL of p24 protein	$1–2
HIV RNA by PCR	Target amplification of HIV-1 RNA via reverse transcription followed by PCR	Reliable to 40 copies/mL of HIV RNA	$75–150
HIV RNA by bDNA	Measurement of levels of particle-associated HIV RNA in a nucleic acid capture assay employing signal amplification	Reliable to 50 copies/mL of HIV RNA	$75–150
HIV RNA by TMA	Target amplification of HIV-1 RNA via reverse transcription followed by T7 RNA polymerase	Reliable to 100 copies/mL of HIV RNA	$225
HIV RNA by NASBA	Isothermal nucleic acid amplification with internal controls	Reliable to 80 copies/mL of HIV RNA	$75–150

[a]Sensitivity figures refer to those approved by the U.S. Food and Drug Administration. [b]Prices may be lower in large-volume settings.

Abbreviations: bDNA, branched DNA; cDNA, complementary DNA; EIA, enzyme immunoassay; TMA, transcription-mediated amplification; NASBA, nucleic acid sequence–based amplification; PCR, polymerase chain reaction.

also increase, particularly when detected by techniques involving dissociation of antigen-antibody complexes. The p24 antigen capture assay has its greatest use as a screening test for HIV infection in patients suspected of having the acute HIV syndrome, as high levels of p24 antigen are present prior to the development of antibodies. Its use as a stand-alone test for routine blood donor screening for HIV infection has been replaced by use of NAT or "fourth-generation" assays that combine antigen and antibody testing. The ability to measure and monitor levels of HIV RNA in the plasma of patients with HIV infection has been of extraordinary value in furthering our understanding of the pathogenesis of HIV infection, in monitoring the response to cART, and in providing a diagnostic tool in settings where measurements of anti-HIV antibodies may be misleading, such as in acute infection and neonatal infection. Four assays are predominantly used for this purpose. They are reverse transcriptase PCR (*RT-PCR*; Amplicor); branched DNA (*bDNA*; VERSANT); transcription-mediated amplification (*TMA*; APTIMA); and nucleic acid sequence–based amplification (*NASBA*; NucliSENS). These tests are of value in making a diagnosis of HIV infection, in establishing initial prognosis, and in monitoring the effects of therapy. In addition to these four commercially available tests, the *DNA PCR* also is employed by research laboratories for making a diagnosis of HIV infection by amplifying HIV proviral DNA from peripheral blood mononuclear cells. The commercially available RNA detection tests have a sensitivity of 40–8100 copies of HIV RNA per milliliter of plasma. Research laboratory–based RNA assays can detect as few as one HIV RNA copy per milliliter, while the DNA PCR tests can detect proviral DNA at a frequency of one copy per 10,000–100,000 cells. Thus, these tests are extremely sensitive. One frequent consequence of a high degree of sensitivity is some loss of specificity, and false-positive results have been reported with each of these techniques. For this reason, a positive EIA with a confirmatory Western blot remains the "gold standard" for a diagnosis of HIV infection, and the interpretation of other test results must be done with this in mind.

In the RT-PCR technique, following DNAse treatment, a cDNA copy is made of all RNA species present in plasma. Because HIV is an RNA virus, this will result in the production of DNA copies of the HIV genome in amounts proportional to the amount of HIV RNA present in plasma. This cDNA is then amplified and characterized using standard PCR techniques, employing primer pairs that can distinguish genomic cDNA from messenger cDNA. The bDNA assay involves the use of a solid-phase nucleic acid capture system and signal amplification through successive nucleic acid hybridizations to detect small quantities of HIV RNA. Both tests can achieve a tenfold increase in sensitivity to 40–50 copies of HIV RNA per milliliter with a preconcentration step in which plasma undergoes ultracentrifugation to pellet the viral particles. In the TMA assay, a cDNA copy of viral RNA is made using primers that contain a promoter sequence for T7 RNA polymerase. T7 polymerase is then added to produce multiple copies of RNA amplicon from the DNA template. It is qualified at 100 copies/mL. The NASBA technique involves the isothermal amplification of a sequence within the gag region of HIV in the presence of internal standards and employs the production of multiple RNA copies through the action of T7-RNA polymerase. The resulting RNA species are quantitated through hybridization with a molecular beacon DNA probe that is quenched in the absence of hybridization. The lower limit of detection for the NucliSENS assay is 80 copies/mL.

In addition to being a diagnostic and prognostic tool, RT-PCR and DNA-PCR are also useful for amplifying defined areas of the HIV genome for sequence analysis and have become an important technique for studies of sequence diversity and microbial resistance to antiretroviral agents. In patients with a positive or indeterminate EIA test and an indeterminate Western blot, and in patients in whom serologic testing may be unreliable (such as patients with hypogammaglobulinemia or advanced HIV disease), these tests for quantitating HIV RNA in plasma or detecting proviral DNA in peripheral blood mononuclear cells are valuable tools for making a diagnosis of HIV infection; however, they should be used for diagnosis only when standard serologic testing has failed to provide a definitive result.

LABORATORY MONITORING OF PATIENTS WITH HIV INFECTION

The epidemic of HIV infection and AIDS has provided the clinician with new challenges for integrating clinical and laboratory data to effect optimal patient management. The close relationship between clinical manifestations of HIV infection and CD4+ T cell count has made measurement of CD4+ T cell numbers a routine part of the evaluation of HIV-infected individuals. The discovery of HIV as the cause of AIDS led to the development of sensitive tests that allow one to monitor the levels of HIV in the blood. Determinations of peripheral blood CD4+ T cell counts and measurements of the plasma levels of HIV RNA provide a powerful set of tools for determining prognosis and monitoring response to therapy.

CD4+ T Cell Counts The CD4+ T cell count is the laboratory test generally accepted as the best indicator of the immediate state of immunologic competence of the patient with HIV infection. This measurement, which can be made directly or calculated as the product of the percentage of CD4+ T cells (determined by flow cytometry) and the total lymphocyte count (determined by the white blood cell count [WBC] multiplied by the lymphocyte differential percentage), has been shown to correlate very well with the level of immunologic competence. Patients with CD4+ T cell counts <200/μL are at high risk of disease from *P. jiroveci*, while patients with CD4+ T cell counts <50/μL are at high risk of disease from CMV, mycobacteria of the *M. avium* complex (MAC), and/or *T. gondii* (Fig. 226-31). Once the CD4+ T cell count is <200/μL, patients should be placed on a regimen for *P. jiroveci* prophylaxis, and once the count is <50/μL, primary prophylaxis for MAC infection is indicated. As with any laboratory measurement, one may wish to obtain two determinations prior to any significant changes in patient management based on CD4+ T cell count alone. Patients with HIV infection should have CD4+ T cell measurements performed at the time of diagnosis and every 3–6 months thereafter. More frequent measurements should be made if a declining trend is noted. For patients who have been on cART for at least 2 years