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MINIREVIEW

Laboratory Tests for Detection of Human Immunodeficiency Virus Type 1 Infection

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SEROLOGIC TESTS FOR INDIRECT DETECTION OF HIV-1 INFECTION: INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is a lymphotropic retrovirus that primarily infects and destroys $CD4^+$ lymphocytes (39). These cells are crucial for the induction and regulation of the immune response. Their progressive depletion by the virus causes irreversible disruption of normal immune function, leading to immunosuppression and the subsequent development of AIDS (38). Early medical intervention reduces the risk of vertically transmitted infection from mother to infant (27a) and delays progression to AIDS (88). This necessitates early detection of the infection, which may be asymptomatic for a prolonged time (70). Infection can be established by direct or indirect laboratory tests. Direct tests detect the presence of the whole virus, its proteins, or its genetic components. They include the p24 antigen capture assay, viral culture, and PCR. Indirect tests detect the presence of antibodies to HIV-1, thus indicating exposure to and infection by the virus. These include enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA), Western blotting (WB; immunoblotting), indirect immunofluorescence assay (IFA), and radioimmunoprecipitation assay (RIPA). This minireview describes the principles, characteristics, and indications for the use of indirect tests.

Antibody (indirect) detection tests comprise screening and confirmatory (supplemental) assays which are characterized by their high degrees of sensitivity and high degrees of specificity, respectively. Screening tests include ELISAs or EIAs and simple and rapid tests. Confirmatory tests include WB, IFA, and RIPA.

ELISA OR EIA

Description. EIA is a qualitative immunoassay characterized by easy performance, high degree of reproducibility, extreme sensitivity, adaptability to automation, and low cost. It was first licensed by the U.S. Food and Drug Administration (FDA) in 1985 to screen donated blood for HIV-1 infection (93). Since then it has also been applied to clinical diagnosis, screening of individuals at risk for infection, and epidemiologic surveillance (21). In 1992, FDA approved another EIA for the simultaneous detection of antibodies to HIV-1 and HIV-2, another AIDS-causing lymphotropic retrovirus.

EIAs are based on different principles: indirect, competitive, and sandwich (29, 114). All EIAs use a solid-phase support (microwells, membranes, beads) onto which various forms of HIV-1 antigens (whole viral lysate, purified viral proteins, recombinant proteins, synthetic peptides, or combinations) are adsorbed. Test specimen (serum, plasma, dried blood spot eluates, urine, saliva) and conjugate are incubated either simultaneously (competitive EIA) or sequentially (indirect EIA) with the solid-phase-adsorbed antigens. The anti-HIV-1 antibodies in the specimen either compete with the conjugate for the immobilized antigens (competitive) or are captured by these antigens, forming antigen-antibody complexes which are then bound by the conjugate (indirect). They are then detected by measuring the intensity (absorbance) of the color formed after adding the proper substrate. The intensity is inversely (competitive) or directly (indirect) proportional to the concentration of HIV-1 antibodies in the test sample. Thus, a sample is considered reactive for HIV-1 antibodies if its absorbance is below (competitive) or above (direct) a certain cutoff point. Fluorescence is also used as an indicator for detection.

Predictive value. HIV-1 EIA is designed to be extremely sensitive in order to detect all truly infected individuals. As a result, false-positive reactivity is likely to occur. Thus, a reactive result may be either truly positive or falsely positive. The probability of being truly positive (positive predictive value [PPV]) is a direct function of sample reactivity and the prevalence of infection in the population tested. In a low-risk population, in which the prevalence of infection is low, the PPV of a weakly reactive EIA is 2% compared with a PPV of 87 to 100% for a strongly reactive EIA. In contrast, in a high-risk population, in which the prevalence of HIV-1 is ≥30%, the PPV of a weakly reactive EIA is 87% compared with a PPV of 99 to 100% for a moderately or strongly reactive EIA (53, 105, 116). On the other hand, the negative predictive value (probability of being truly negative) of a nonreactive EIA is 75 to 100% in a high-risk population and close to 100% in a low-risk population (60, 105).

FPRs. Contemporary EIAs produce fewer false-positive results (FPRs) than earlier ones as a result of their enhanced sensitivities and specificities (2, 89) (Table 1). EIAs that use whole viral lysate as a source of antigen contain numerous and a wide range of antigenic sites representing the majority of HIV-1 proteins. This ensures detection of antibodies to different HIV-1 subtypes and reduces the chance of missing a variant. It compromises the specificity of the assay, however, resulting in FPRs. Sera containing antibodies that recognize an epitope shared by HIV-1 and other viruses or bacteria or antibodies that bind human leukocyte antigens and other host cell components present in the lysate are falsely reactive. Such reactivity has been circumvented by propagating the virus in cells lacking human leukocyte antigens, testing each specimen against a noninfected cell lysate, or using recombinant proteins

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TABLE 1. Causes of FPRs by EIA

Cause of FPRs (reference[s])
Performance and technical errors (104)
Mislabeling of samples or wells
Carryover or cross contamination (76)
Variability in test kits (60)
Heat treatment of samples (24, 59)
Purity of HIV-1 antigens
Presence of anti-HIV-1 antibodies in:
Noninfected babies born to infected mothers
Recipients of unscreened immunoglobulins (45, 48, 68, 109)
Recipients of HIV-1 trial vaccines
Presence of antibodies reactive with:
Human leukocyte antigens or other cellular components, such as those observed in
Multiparous women and polytransfused patients (10, 55, 67, 87, 101)
Patients on chronic hemodialysis (92, 111)
Patients with autoimmune diseases (4, 16, 87, 108)
Anti-idiotype antibody (conjugate) (26, 91)
Recipients of influenza virus and hepatitis B virus vaccines (26, 71, 75)
Patients infected with herpes simplex virus 2 (26)
Epitope shared by HIV-1 and other retroviruses, rabies virus (91), or *Mycobacterium leprea* (61)
Others
Congenital bleeding disorders (107)
Alcoholic hepatitis (82)
Hematologic malignancies (108)
Positive reagin test (42)

or synthetic peptides as a source of antigen. Recombinant proteins are genetically engineered segments that represent highly conserved immunodominant regions of the *env* and/or *gag* proteins and that contain several antigenic sites. Synthetic peptide antigens consist of short amino acid sequences (10 to 40 amino acids) representing few epitopes of these sites. While synthetic antigen preparations have consistent and reproducible purities, recombinant preparations may contain host cell (bacterial or yeast) contaminants that cause few FPRs. Both antigen types are devoid of human cellular components. They can be produced in large quantities, at low cost, and with no biohazardous consequences. Also, they can be incorporated onto a solid support at a high density, thereby increasing the signal-to-noise ratio and thus the sensitivity of the assay (34a). Compared with whole viral lysate, the advantages conferred by recombinant and synthetic peptide antigens include decreased numbers of FPRs, increased sensitivity, and earlier detection of seroconversion. As a result, repeat and confirmatory tests are minimized and the cost is reduced.

False-negative results. Recombinant and synthetic antigen-based EIAs are very sensitive and specific for the HIV-1 subtypes found in the United States and Europe. However, some react poorly with African sera (4a), and not all could detect the new highly divergent HIV-1 subtype O all the time (73a, 103) (Table 2). This may be due to an inadequate amount or inappropriate sequence of the antigen used, low-affinity antibody in the sera tested, low antibody titer, or inherent features of the assay (73a). Although failure to detect subtype O has major consequences for the safety of blood supplies, it is not a major concern in the United States because subtype O is endemic in Cameroon and Gabon and has not been reported worldwide, donors who have originated from or visited those countries are excluded from donating blood for several years, and all high-risk donors are excluded as well (103).

The emergence of HIV-1 subtype O raises concerns about the ability of currently available EIAs to detect additional as yet unidentified subtypes and subtypes that may emerge in the future. It also underscores the importance of issues considered by the FDA for approving tests based on synthetic or recombinant antigens. The major issue focuses on the ability of EIAs with limited antigenic representation to detect divergent variants that may potentially emerge as a result of the extensive variability of HIV-1. This variability is more pronounced in the *env* than in the *gag* and *pol* regions. EIAs based on synthetic peptide antigens use a peptide containing few epitopes that represent a highly conserved immunodominant region of the *env* glycoprotein (gp41). Thus, a genetic drift in the region may induce antibodies that may escape detection by the narrow range of epitopes used in this assay. In order to reduce the number of false-negative results because of this, FDA has asked manufacturers to include in the assay additional peptides from the *gag* or *env* region that are also immunodominant and highly conserved. In addition, manufacturers have been asked to test sera derived from different geographical locations and large numbers of randomly selected positive serum samples in the process of evaluating recombinant and synthetic antigen-based assays.

Resolving FPRs and false-negative results. Most FPRs produced by EIA are resolved by confirmatory testing. However, if the result remains inconsistent with the patient's history and the clinical findings, retesting of the specimen by the same or a different EIA, performing PCR and/or culture, or retesting a fresh sample can be done. Similar approaches may be used to resolve false-negative results.

Testing algorithm. The Centers for Disease Control and Prevention and other public health services in the United States recommend performing EIA initially on a single specimen (21, 22, 50) (Fig. 1). A nonreactive specimen requires no

TABLE 2. Causes of false-negative results by EIA

Cause of false-negative results (reference[s])
Performance and technical errors (104)
Pipeting error (122)
Mislabeling of samples or wells
Variability in test kits
Decreased intensity of EIA reaction by
Powder from powdered gloves (69)
Storing of samples in serum separator tubes (81)
Biologic, pathologic, and pharmacologic determinants
Window (preseroconversion) period (78, 117)
Delayed antibody synthesis in infants
Diminished immune response because of
HIV-1-related immune dysfunction (79)
Immunosuppressive therapy (1, 79, 118)
Concurrent infection with Epstein-Barr virus or cytomegalovirus (80)
Congenital or drug-induced hypogammaglobulinemia (12, 66)
Formation of antigen-antibody complexes (46)
Sensitivity and specificity of the assay
Infection by HIV-1 subtype O (103)
Sampling prior to immunoglobulin M to immunoglobulin G class switching (5, 119)
Hemodilution (20)
Nondenatured antigenic epitopes (96)
Limited antigenic determinants

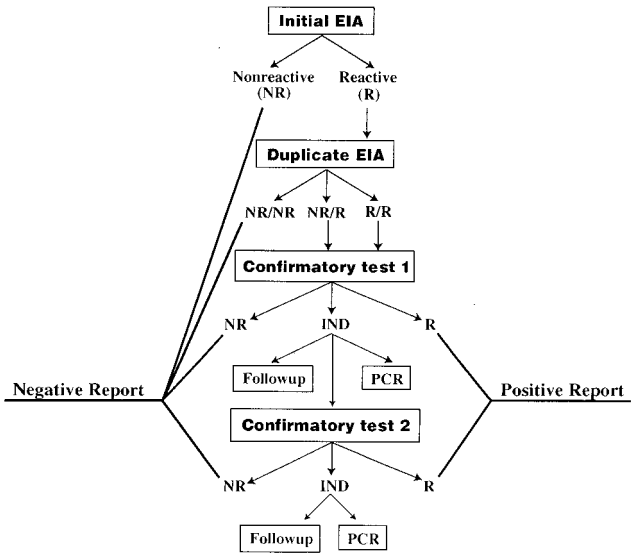

FIG. 1. HIV-1 antibody testing algorithm. This algorithm is based on the recommendations of the Centers for Disease Control and Prevention and Association of State and Territorial Public Health Laboratory Directors, FDA-licensed kits, and the reported literature. R, reactive; NR, nonreactive; IND, indeterminate.

immediate further testing, while a reactive specimen should be retested in duplicate in order to reduce the likelihood of an FPR. If both duplicates are nonreactive, then the result is negative only if the patient belongs to a low-risk group; otherwise, a fresh sample should be tested in 3 to 6 months. On the other hand, if either or both of the duplicates are reactive, the specimen is repeatedly reactive for the presence of HIV-1 antibodies. Its reactivity must then be confirmed by a more specific test (WB or IFA), which allows the laboratory and the clinician to distinguish between false-positive reactivity and true-positive reactivity. Because FPRs are likely to be produced by EIA and because a positive HIV-1 result has a tremendous psychological and social impact, public health services in the United States recommend reporting a positive result only after a repeatedly reactive EIA has been confirmed (22). A specimen that is repeatedly reactive by EIA and reactive by a confirmatory test is positive for the presence of anti-HIV-1 antibodies (3, 21). If the confirmatory test is negative, the patient most likely is not infected (3, 15) and may not present a risk for the transmission of HIV-1 infection (37, 72). If it is indeterminate it can be resolved by using another confirmatory test (3, 115), performing PCR and/or culture (36), or retesting in 6 months (3).

Blood banks incorporate HIV-2 screening into their algorithms, as mandated by FDA (19a). Either two separate EIAs, one each for HIV-1 and HIV-2, or a single EIA for HIV-1 and HIV-2 combined is used initially. Donated units that are nonreactive or reactive by initial screening but nonreactive on both repeat duplicate screenings are considered negative and are put into use. Repeatedly reactive units are discarded. However, confirming their infection with HIV-1 and/or HIV-2 is necessary. Confirmatory testing for HIV-1 is performed first since in the United States HIV-1 is more prevalent, HIV-2 infection is rare, and a licensed HIV-1 and HIV-2 WB test is not yet available. If the result is positive, the donor is notified and the recipients of previous donations are traced. If it is negative or indeterminate, ruling out infection with HIV-2 should follow. Since no licensed confirmatory test for HIV-2 is available, performing a second EIA for HIV-2 different from the initial one is recommended. A nonreactive result indicates a remote likelihood of infection, while a repeatedly reactive result confirms it. A donor who is confirmed to be positive for HIV-1 and/or HIV-2 is permanently deferred from donating. A donor who is HIV-2 negative and whose HIV-1 status is repeatedly reactive by EIA but either negative or indeterminate by a confirmatory test is reenrolled as a donor after at least 6 months if all tests become negative.

Applicability. Currently available EIAs have excellent performance, with analytical sensitivities and specificities that exceed 99 and 98%, respectively (25). However, some are more applicable for blood screening, while others are better suited for clinical diagnosis. Features of the former EIAs include high negative predictive values to ensure that negative blood units are not likely to be infectious, maximum sensitivity to protect recipients, and optimal specificity to prevent deferral of donors, inaccurate notification, and wastage of blood. EIAs with high PPVs and high degrees of specificity are important for clinical diagnosis. They ensure that a positive test is indicative of HIV-1 infection.

Alternative testing sequence. Because of the complexity, cost, tedium, nonuniform result interpretation, and frequent indeterminate results of WB, the use of IFA or EIA as a confirmatory test has been suggested (41, 98). The use of IFA has already been implemented by some laboratories. Recombinant- or synthetic peptide-based EIAs are comparable to WB in their specificities but are simpler and cheaper and can be performed faster. Algorithms that use two sequential EIAs that differ either in principle or in antigen source provide diagnostic accuracy similar to that of EIA-WB (111a). However, the use of EIA with or without WB is impractical in developing countries because of the scarcity of well-equipped laboratories and the shortage of resources. The sequential use of two instrument-free, rapid and simple screening tests (RSTs) that differ either in principle or in the source of antigen (6, 11, 84, 86) has been suggested in developing countries. This algorithm has a diagnostic accuracy comparable to that of EIA-WB (11, 86), provides 100% sensitivity and specificity when the more sensitive assay is performed first (112), and is cheaper (112). However, its performance is inferior to that of the EIA-WB when it is performed by untrained personnel, in the absence of quality control practices, and under field conditions (84).

The World Health Organization (WHO) recommends three different testing strategies on the basis of testing objective, prevalence of infection, and the presence or absence of symptoms (124). Strategy I is recommended for screening blood in any population regardless of the prevalence of infection. Each specimen is tested by a single EIA or RST. Reactive units are discarded. Strategies II and III are recommended for clinical diagnosis. Strategy II is applied to all symptomatic individuals in any population regardless of the prevalence of infection and to asymptomatic individuals only in populations in which the prevalence of infection is $>10\%$. It involves the use of strategy I for initial testing. Reactive sera are then retested by a second EIA or RST that differs from the first one either in principle or in the source of antigen. Samples reactive in both tests are regarded as positive. Those that are nonreactive in either test are regarded as negative. Strategy III is applied to asymptomatic individuals in populations in which the prevalence of infection is $\leq 10\%$; serum is initially tested by strategy I, and if the sample is reactive, strategy II is applied; if the result is again reactive, it is then retested by a third EIA or RST different from the first two in principle or antigen preparation. A sample is regarded as positive if it is reactive by the three tests,

negative if it is nonreactive by either of the first two tests, and equivocal if it is reactive by the first two tests but nonreactive by the third one. For surveillance purposes, strategies I and II are recommended in populations in which the prevalences are >10 and ≤10%, respectively.

RAPID AND SIMPLE SCREENING TESTS

Description. RSTs are referred to as rapid since they can be performed in minutes instead of hours and are referred to as simple because they require minimal technical skills and need no instruments and the results can be interpreted visually. They can be used as a screening alternative to conventional EIAs in developing countries, field projects, and emergency situations. In the United States, their implementation in emergency departments is hindered by unresolved ethical and practical issues (62).

RSTs are qualitative tests, the majority of which are based on particle agglutination and dot blot immunoassay principles. Agglutination tests (63, 95, 97) involve the mixing of serum or whole blood with HIV-1 antigen-coated latex, gelatin, polystyrene particles, or erythrocytes. HIV-1 antibodies in the sample cross-link antigens on separate particles, bringing them in proximity to each other and resulting in their aggregation or agglutination. According to some reports, latex agglutination assays have sensitivities and specificities comparable to those of conventional EIA, and their results exhibit complete concordance with those of WB (43, 49, 95, 97). Others have demonstrated low degrees of sensitivity (92%) and interobserver variability (20%) in interpreting the results, even when the tests are performed by trained and skilled personnel (54).

In dot blot immunoassays, HIV-1 antigens are adsorbed in a circular (dot) manner either to a membrane surface or to microparticles trapped within a membrane. Immunoassays that use microparticles are referred to as solid-phase capture. Comparative evaluation of six rapid assays (three solid-phase capture assays, two dot blot assays, one latex agglutination assay) showed that solid-phase capture immunoassays have the highest degrees of sensitivity (>99%) and specificity (>92%) and are the easiest to perform and their results are the easiest to interpret (77). Moreover, interpretation of the results is consistent among observers or by the same observer (49).

IMMUNOBLOTTING OR WESTERN BLOTTING

Description. WB is a qualitative immunoassay that is used to confirm the presence of antibodies to HIV-1 in a sample that is repeatedly reactive by EIA. It also identifies individual HIV-1 proteins against which these antibodies are directed. Compared with EIA, WB is more complex, time-consuming, expensive, and specific and has a lower PPV when it is performed separately. The PPV of WB performed sequentially with EIA exceeds 99% with samples from low- or high-risk populations (31). Thus, WB is not recommended for use in screening and should only be performed sequentially with EIA on repeatedly reactive samples.

Similar to EIA, WB detects antibodies to HIV-1 by using anti-human immunoglobulin enzyme conjugate and solid-phase immobilized HIV-1 proteins (antigens). However, it differs from EIA in the ways in which these proteins are distributed. In EIA, mixed proteins are randomly adsorbed to the solid phase. In WB, they are first electrophoretically separated into discrete bands according to their size and are then transferred to nitrocellulose membranes. The membranes are cut into strips and are then used to detect and identify antibodies to HIV-1-specific proteins. Each strip serves as a solid matrix on which an indirect EIA procedure is performed. Briefly, separate strips are serially incubated with (i) three controls (nonreactive, weakly reactive, and strongly reactive) or a test sample, (ii) a conjugate, and (iii) a substrate. Antibodies in the sample and in the reactive controls bind their corresponding antigens on the strips, forming antigen-antibody complexes. Then, the enzyme conjugate binds to these complexes and converts a chromogenic substrate into a colored product which precipitates in situ (where the complexes are located) in the form of bands. The intensity of each band is visually evaluated by comparing it with a reference band (gp41, gp120, or p24) on the weakly reactive control (9, 17, 89a). Also, the identity of each band is established by using the strongly reactive control strip as a reference. This strip exhibits all major protein bands encoded by the three structural genes of HIV-1: core proteins p55, p24, and p18 encoded by *gag*; envelope glycoproteins gp160, gp120, and gp41 encoded by *env*; and polymerase enzyme proteins p66, p51, and p31 encoded by *pol*.

Interpretation. On the basis of the banding pattern (number and type of bands) seen on each strip, the WB result is interpreted as positive, atypical or indeterminate (WBi), or negative. Different interpretive criteria have been established by different organizations. With the exception of WHO, all organizations agree that WB is negative if it exhibits no bands at all and indeterminate if the bands present do not meet the criteria for positivity. (WHO considers a WB result to be negative only if non-HIV-1 bands are present). The criteria for positivity are not uniform, and they have been constantly evolving with time. Currently, in the United States the most commonly used criteria are those established by the Association of State and Territorial Public Health Laboratory Directors (50) and later adopted by the Centers for Disease Control and Prevention (22, 23) and also approved by FDA (9, 17, 89a). By these criteria, a WB result is positive if it exhibits at least any two bands of p24, gp41, and gp120/160. Reactivity to the following bands renders WB positive by other organizations: three bands, one from each gene product (*gag*, *pol*, *env*), by the American Red Cross; p24 or p31, in addition to gp41 or gp120/160, by the Consortium for Retrovirus Serology Standardization (28); and two envelope glycoproteins by WHO (123). A sample that contains antibodies to all major viral proteins is positive by all criteria, but a sample containing antibodies to few proteins may be indeterminate by one organizational criterion but positive by another one. Therefore, it is important to evaluate a WBi result in conjunction with the clinical history of the individual. In general, interpreting a WBi result for a patient at high risk of infection and manifesting AIDS-related symptoms is easy. However, it becomes a challenge to the physician and a source of great stress to a patient with low risk in the absence of symptoms.

Resolving WBi results. WBi results may be resolved by IFA and/or PCR or by follow-up testing in 3 to 6 months. CDC recommends that a consistent and stable WBi result for at least 6 months be considered negative for a low-risk individual with no clinical symptoms or other indicators (23). Healy et al. (52) proposed classifying WBi patterns by their reactivities to certain bands in order to reduce the number of follow-up tests needed for those with WBi results and to report clear results. In the absence of clinical or epidemiologic indications, patterns exhibiting no reactivity to *env* glycoproteins or p24 (with or without p18) are not due to HIV-1 infection, do not need follow-up testing, and could be reported to be negative. On the other hand, samples with patterns exhibiting reactivity to p24 or *env* glycoproteins are most probably infected with HIV-1 and require additional tests to resolve the infection status. If these additional tests are not informative, follow-up testing in

TABLE 3. Conditions associated with WBi result

Condition (reference[s])
Incomplete generation or loss of antibodies
Early seroconversion (33, 73, 106)
Late-stage disease (8)
Massive proteinuria (85)
Passive transfer of antibodies from:
Infected mothers to noninfected children (18)
Unscreened immunoglobulin preparations (48, 68, 109)
Cross-reactivity with:
Normal cellular constituents: nucleoproteins (99) and HLA (35)
Other retroviruses: human T-cell leukemia virus type 1 and HIV-2 (33)
Bacteria (*Mycobacterium leprae* [61])
Interfering factors or sample preparation
In vitro hemolysis, elevated bilirubin, rheumatoid factor (17)
Heat inactivation (24, 47)
Antibodies generated by influenza virus vaccine (75)
African sera (34a)
Disease states
Polyclonal gammopathies (17)
Systemic lupus erythematosus (4)

3 months is indicated. This strategy is used throughout Australia.

Significance, prevalence, and causes of WBi results. Follow-up studies of donors and recipients of blood with WBi results found no evidence of HIV-1 infection in donors who tested negative by PCR, viral culture, p24 antigen, or a combination of these tests or in the recipients (44, 113). These and other studies (33, 58, 72) have demonstrated that a low-risk individual whose EIA result is either reactive or nonreactive and whose WB result is persistently indeterminate probably poses no risk for transmitting HIVs. However, excluding blood donors exhibiting such a profile from donating blood is highly recommended (30, 33). Others have shown that the significance of a WBi result is dependent on the observed pattern. Patterns exhibiting reactivity to p24 or *env* glycoproteins are most probably due to HIV-1 infection, while those exhibiting reactivity to other bands are not (52, 65).

Most samples with WBi patterns (60 to 100%) exhibit reactivity to one or more *gag* proteins (p18, p24, p55) (32, 33, 36, 56, 65, 113); 25 to 50% of the WBi patterns are due to p24 (34). Few are due to envelope glycoproteins (32, 83). The reactivity is independent of reactivity by EIA. The patterns of samples with WBi results and EIA-negative and EIA-positive results are similar (44, 72) and occur at rates of 3 to 32% (17, 23, 44, 83) and 8 to 48% (56, 64, 65, 74), respectively. The overall rate of an EIA-negative WBi result (0.13%) or an EIA-positive WBi result (0.005 to 0.13%) (34, 65) exceeds that of FPRs of EIA and WB combined (0.0007%) in a similar population (13, 74). This substantiates the notion that WB must be used as a confirmatory test for specimens that are already repeatedly reactive by EIA.

The conditions associated with a WBi result are listed in Table 3. Some WBi patterns are nonspecific (44, 72) and are stable with time; others represent a transient stage in the progression of the disease. The rate of conversion of a WBi result to a positive result by WB is 1 to 5% in blood donors (19, 34, 65). Autoimmune reactivity has been observed in 50% of blood donors with a WBi result (33).

FPRs and false-negative results. Erroneous results of serial EIAs and WBs are very rare, although they occur (7, 14, 30, 100). They are attributed to the same conditions that cause FPRs and false-negative results in EIAs. A false-positive WB result has recently been reported in a patient with systemic lupus erythematosus on the basis of negative viral culture, PCR, and p24 antigen results (57). However, the report was later withdrawn after HIV-1 infection was confirmed by viral culture and PCR by using conditions slightly different from conventional ones (94). False reactivity to gp41 has also recently been described in blood donors with no risk factor for HIV-1 infection (51, 102). This underscores concerns that have been raised regarding the interpretation of positive WB assays exhibiting envelope bands only. In some WB assays, tetrameric and trimeric forms of gp41 comigrate with gp160 and gp120, respectively, resulting in an apparent reactivity to gp41 and gp120/160 (40, 126), which is a positive result by some criteria.

INDIRECT IMMUNOFLUORESCENCE ASSAY

Description. IFA is a qualitative immunoassay recently licensed by FDA for use in screening for HIV-1 infection, confirming the presence of anti-HIV-1 antibodies, and resolving WBi results. Compared with WB, it is simple, fast, and inexpensive, has sensitivity and specificity comparable to those of WB, and exhibits 100% concordance with WB (110). Although its major advantage is the low frequency of indeterminate results, its use is less favorable because it needs an expensive microscope, requires expertise for reading and interpreting the results, does not provide permanent test records, and does not identify individual HIV-1 proteins to which antibodies are directed.

IFA and indirect EIA share the same principle (detection of HIV-1 antibodies by using labeled anti-human immunoglobulin conjugate and immobilized HIV-1 antigens). However, they differ in the form of antigen and the type of indicator used. In IFA, inactivated HIV-1-infected cells expressing HIV-1 antigens are fixed on a slide and serve as the solid matrix. Uninfected cells fixed on the same slide serve as a negative control. The cells (infected and uninfected) are first incubated with test serum and are then incubated with a fluorescence-labeled conjugate. The pattern and intensity of the fluorescences that they exhibit are evaluated for each sample by using a UV light-equipped microscope (115).

Interpretation. IFA results are interpreted as positive, negative, or indeterminate. It is positive when infected cells exhibit a specific fluorescence pattern with an intensity significantly different from that of uninfected cells. The absence of specific fluorescence from both infected and uninfected cells indicates a negative result, while similar fluorescence patterns with similar intensities indicate an indeterminate result. Patterns indicating an indeterminate result are nonspecific and may be observed in patients with systemic lupus erythematosus, autoimmune diseases, and severe paraproteinemia (115).

RADIOIMMUNOPRECIPITATION

Description. RIPA is a complex, expensive, and time-consuming confirmatory test whose use is restricted to specialized laboratories. It was often used to resolve WBi results before IFA and PCR became available. It involves growing HIV-1-infected cells in the presence of radiolabeled amino acids, lysing these cells, and incubating the sera to be tested with whole viral lysate. The antibodies in the test sera bind radiolabeled viral antigens, forming radiolabeled antigen-antibody complexes. These complexes are then immunoprecipitated and

separated by electrophoresis, and the labeled proteins are detected by autoradiography. The bands on the autoradiographs correspond to viral proteins recognized by the anti-HIV-1 antibodies present in the test sample. RIPA is more sensitive than WB for detecting antibodies to the *env* glycoproteins gp120 and/or gp160, while WB is more sensitive than RIPA for detecting antibodies to low-molecular-weight proteins including gp41 and core proteins (27).

LIMITATIONS OF ANTIBODY DETECTION METHODS

In all antibody detection methods, a negative result does not exclude the possibility of HIV-1 infection. Recent infection in a patient who belongs to a high-risk group, a patient who has received a blood transfusion, or a patient who is immunosuppressed cannot be ruled out (78). Moreover, assays that detect immunoglobulin G (IgG) antibodies do not distinguish between transplacentally acquired maternal immunoglobulins and those of the infant. Thus, they are not diagnostically useful for infants less than 18 months old. Assays specific for detecting immunoglobulins incapable of crossing the placenta (IgA and IgM) have been developed (120). However, IgM detection has some limitations, while IgA detection is more promising and has been developed for EIA, WB, and dot blotting. The sensitivity of IgA assays is close to 100% for infected infants who are 6 months or older (90).

CLOSING REMARKS

Current HIV-1 antibody detection methods have been effective in reducing the risk of transfusion-associated AIDS and accurately diagnosing infection in asymptomatic individuals. The rate of detection of HIV-1 infection during early seroconversion has increased from 89% with second-generation EIAs to 94% with third-generation EIAs (125). Also, the prevalence of confirmed HIV-1 infection in blood donors has dropped to less than 5 per 100,000 units (121). However, continued efforts to increase the sensitivities and specificities of these tests and to develop new or supplemental tests are critically needed in order to detect potentially divergent subtypes, completely eliminate all risks of transmitting HIV-1 in association with blood transfusion, and accurately diagnose neonatal infections. A second part of this minireview will discuss how direct testing for HIV-1 supplements indirect testing in achieving these goals. It will also discuss the benefits and pitfalls of using direct tests.

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