Indeterminate Results in Blood Donor Testing: What You Don't Know Can Hurt You

Roger Y. Dodd and Susan L. Stramer

ALTHOUGH current tests for transfusion-transmissible infections have excellent performance characteristics, they are used to test populations with very low prevalence rates. As a consequence, the positive predictive value of these tests tends to be low. This was first recognized when sensitive radioimmunoassay tests were introduced to detect hepatitis B surface antigen (HBsAg). Although the apparent yield of this test was about 4-fold greater than that for the counterimmunoelectrophoresis tests then in use, it soon became apparent that most of the additional reactive samples did not represent true positives. Immunologic blocking assays were developed and implemented so that donors could be given accurate information about their infection status. For most tests implemented since that time, efforts have been made to develop confirmatory or supplementary tests.

Tests for antibodies to infectious agents do not lend themselves to confirmation by simple inhibition or blocking techniques, so other approaches have been used. The Western blot, a research test, was introduced to supplement the human immunodeficiency virus (HIV) antibody test, and it established an unfortunate precedent. A number of other tests that were introduced subsequently also used Western blot technology (ie, human T cell lymphotropic virus [HTLV]-I, HTLV-II) or the somewhat similar strip immunoassay (SIA) for hepatitis C virus (HCV). The 2 major difficulties with these approaches have been developing clearly defined criteria for interpretation and the fact that some outcomes of these tests can occur among both those who do and those who do not have true infection. Such patterns of reactivity are termed indeterminate. Thus, it is not possible to give a donor a clear message about the presence or absence of infection in the face of such a result. As will be seen below, there are circumstances in which most results from so-called confirmatory tests are actually indeterminate. In addition, the term "indeterminate" has been used to define a number of test outcomes that appear to have no relationship whatsoever to infection.

This review discusses the frequency and implications of indeterminate results in blood donor screening and offers some suggestions for minimizing the problems. Necessarily, it presents the situation that is current in the United States, where regulatory requirements define many of the policies and procedures in use. Thus, any recommendations are presented only as a basis for further discussion or development and are not intended to circumvent current regulated procedures. Other tests and approaches may be available, or in routine use, in other parts of the world, where, as a result, the situation may be simpler and more satisfactory. It is suggested that there should be new confirmatory strategies that eliminate, rather than supplement, subjective tests, such as immunoblots and immunofluorescence.

IMPACT OF INDETERMINATE PATTERNS

There is a clear scientific and ethical rationale for confirming the results of blood donor screening tests. In general, only a minority of individuals with reactive screening test results are (or have been) infected with the relevant pathogen. Such individuals should be advised of their status so that they may seek medical care and take measures to prevent infecting others. Equally, an individual with a false-reactive screening test result should be reassured that he or she is not infected. As is discussed in depth below, many (often most) supplementary test results among donors are indeterminate (Table 1). This outcome frustrates the need to provide meaningful information. Yet the results of screening tests must be transmitted to the donor, because regulatory requirements do not permit any further blood donation after a repeatedly reactive screening test result, at least until a specific "reentry" testing algorithm has been completed.

From the Transmissible Diseases Department, American Red Cross, Jerome H. Holland Laboratory, Rockville, MD; and the American Red Cross, National Confirmatory Testing Laboratory, and American Red Cross National Reference and Testing Laboratories, Gaithersburg, MD.

Address reprint requests to Roger Y. Dodd, PhD, Head, Transmissible Diseases Department, American Red Cross, Jerome H. Holland Laboratory, 15601 Crabbs Branch Way, Rockville, MD 20855.

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Table 1. Results of Confirmatory Testing Among Voluntary Blood Donors, American Red Cross, 1996-97 (N = 3.27 × 10^6)

Marker	Number (%) EIA Repeat Reactive*	Number (%) of EIA Repeat Reactive Samples With Confirmatory Results of:		
		Number (%)† Positive	Number (%) Negative	Number (%) Indeterminate
Anti-HIV	2,660 (0.082)	208 (7.8)	1,245 (46.8)	1,207 (45.4)
HIV-1 p24 Antigen‡	1,625 (0.024)	134§ (8.2)	NA	1,491 (91.8)
Anti-HTLV-I	3,581 (0.11)	323 (9.0)	692 (19.3)	2,566 (71.7)
Anti-HCV (3.0)	6,032 (0.19)	3,397 (56.3)	1,423 (23.6)	1,212 (20.1)
HBsAg	1,456 (0.045)	1,001 (68.8)	455 (31.2)	NA

*Percentage figure is percentage of all donations found repeat reactive in screening test.

†The percentage is the positive predictive value of the test.

‡Denominator for HIV p24 antigen is approximately 6.75 × 10^6.

§Includes 57 false-positives (RNA and follow-up negative), 73 with anti-HIV and a final yield of 4 anti-HIV negative, RNA positive window case donations.

Presenting blood donors are aware that they will be tested for a variety of infections, including HIV. Clearly, most such presenting donors are aware of their own status with respect to the presence or absence of risk factors associated with these infections.[1-3] Informal reports and studies suggest that donors with true positive test results do not generally appear to be overly surprised when they receive this information. In contrast, a donor with an indeterminate result is often alarmed, surprised, and distressed on learning about the equivocal outcome of the test (Kleinman, personal communication, 1999). Indeterminate results are only rarely associated with specific risk factors—indeed these reactions appear to occur randomly. Furthermore, the message that is presented is one of uncertainty. In some cases, an indeterminate result may be resolved only by subsequent testing after a waiting period, and in other cases, there may never be a satisfactory resolution. Finally, in most cases, current regulatory guidance in the United States requires indefinite deferral of a donor with an indeterminate result.

There have now been many years of experience with confirmatory and supplementary testing. Out of necessity, much information about the significance of indeterminate patterns has emerged. Some indeterminate patterns are never associated with any evidence of infection, based on testing for viral genomic material and on follow-up. Examples would include almost any HIV blot lacking an *env* or p24 band[4] or the presence of only an NS5 band in an HCV SIA 3.0 pattern.[5,6] However, it does not appear that any attempt has been made to consider reclassifying such patterns as negative.

HIV 1 AND 2

The history of blood donor testing for HIV illustrates many of the issues relating to indeterminate test results. Thus, this area is treated in rather more depth than are other tests. The original recognition of HIV (then known as HTLV III) in the United States was reported in 2 papers, 1 of which detailed the isolation and culture of the virus,[7] whereas the other reported on the development of an antiglobulin enzyme immunoassay (EIA) for anti-HIV.[8] The results of this developmental EIA were confirmed by Western blot, thus setting the scene for the eventual acceptance of the Western blot as the dominant supplementary test format for HIV antibodies.

The Western blot procedure involves a number of different steps. First, lysed, purified virus is electrophoresed on polyacrylamide gel, in the presence of sodium dodecyl sulfate. Thus, individual viral proteins, glycoproteins, and peptides are separated essentially according to their molecular weights. The separated proteins are then transferred, or blotted, onto nitrocellulose paper. The paper then may be dried, and the pattern of proteins is retained on the paper. A single nitrocellulose paper blot is then cut into strips along the direction of current flow. A strip then represents a single test. To perform a test, a strip is exposed to a diluted sample of the serum to be evaluated. Antibodies, if present, adhere to the proteins that bear the appropriate epitopes. After a wash step, the antibodies may be detected by the use of an antiglobulin/enzyme conjugate preparation. The complex is visualized by means of a chromogenic substrate that yields an insoluble reaction product. The blot is read by comparing the band positions and densities with those from strongly and weakly positive controls, run at the same time. In effect, the Western blot procedure permits separation and enumeration of different antibody specificities within the same sample, thus providing more information than an

EIA test and differentiating true antiviral reactivities from false-positive reactants.[9]

Proteins that are generally considered to contribute to the diagnostic properties of the HIV-1 Western blot are p17, p24, and p55, referred to as core or *gag* gene products; the p31, p51, and p66 polymerase (*pol*) gene products; and the envelope (*env*) glycoproteins gp41, gp 120, and gp 160.[10] In general, a sample from a chronically infected but asymptomatic individual will eventually demonstrate the presence of antibodies to each of these gene products, and the blot is readily interpreted. However, during the early stages of evolution of the immune response (and in some other, limited circumstances), only partial patterns will be seen. Current experience suggests that the earliest antibodies to appear and be detectable by most blots are those to the *env* glycoproteins and to p24.[11] It has not proved easy to define minimal criteria for infection. Currently, in the United States, minimal criteria used for Food and Drug Administration (FDA)-licensed HIV-1 Western blots are the presence of at least 2 bands at the following peptide locations: p24, gp41, gp120/160—the latter essentially read as one band. To contribute to a positive reading, the band must have a greater visible intensity than that exhibited by the p24 band of the weak positive control.[10] Conversely, to be read as negative, the FDA-approved instructions require that there be no visible bands on the strip. Any band pattern that does not qualify as positive or negative must be interpreted as indeterminate. Although this appears to be quite straightforward in concept, it is far from simple in practice.

At least until the advent of reliable genome-amplification procedures for HIV, there has been no "gold standard" for HIV infection. Consequently, at least in the early days of testing, there was no clear rationale for defining the minimal criteria for a positive blot pattern. The criterion described above (developed by the Centers for Disease Control and Prevention [CDC] and by the Association of State and Territorial Public Health Laboratory Directors [ASTPHLD])[10,12] has been endorsed by the FDA. It involves a balance between sensitivity and specificity but favors sensitivity. Consequently, false-positive blot results have been documented[13,14] and may represent as many as 10% of all HIV-positive interpretations among donor populations.[15,16] Other criteria (such as those that require inclusion of a *pol* band, typically p31) are more specific but correspondingly less sensitive. Although the CDC/ASTPHLD criteria permit some false-positive notifications, it is now possible to resolve these by the appropriate use of HIV p24 antigen testing, along with genome amplification for HIV RNA.[17,18] However, in the past, these tests were not routinely used and, particularly with the less sensitive blot criteria, some (but by no means all) of those individuals whose samples gave particular indeterminate patterns were actually in the early stages of HIV seroconversion. The only way to determine whether this was the case was to examine subsequent samples from the same individual. The original advice provided by CDC (and still in force) suggested that an individual with an indeterminate blot pattern was "almost certainly not infected" with HIV if the indeterminate pattern did not evolve over a period of 6 months. It is now clear that this period was grotesquely long (see below). A critique of interpretive criteria published in 1990 is as true today as it was then.[4]

Unfortunately, at least 4 other situations lead to indeterminate results in an HIV-1 Western blot. First, on rare occasions, an individual infected with HIV-2 will generate an indeterminate pattern in an HIV-1 blot. Second, there are naturally occurring antibodies that react with epitopes on viral proteins. This seems to be particularly true for p24 and for gp41.[14] Careful studies have shown that such reactivities are usually restricted to a single epitope on the protein, whereas antibodies stimulated by infection react to multiple epitopes. Third, there are nonviral proteins on the blot, and antibodies to these may occur. Because they create a band, regulatory imperatives require that such bands be read as indeterminate. Finally, blots occasionally have irrelevant debris or staining that may be distinguished from a band, but that nevertheless have to be read as indeterminate if the debris might hide a legitimate band. Note that the latter 2 situations derive from regulatory, rather than medical or scientific, concerns.

A further complication arises from the current FDA-approved algorithm for use of the combination assays for HIV-1 and HIV-2. A sample with a repeat reactive screening test that proves to be nonreactive or indeterminate in supplementary assays for HIV-1 antibodies requires further testing by a licensed HIV-2 EIA. This too may give a reactive result, which must be reported as HIV-2 positive. There is no licensed blot for HIV-2, and

those blots that are available generate significant proportions of indeterminate outcomes. Table 2 reports the American Red Cross experience and shows that, among 25,288 samples that were negative in an HIV-1 Western blot, 194 samples were repeat reactive in an HIV-2 EIA, and among these, 52 (or 26.8%) were indeterminate in an HIV-2 blot. Among the 17,161 HIV-1 indeterminate samples, 219 were repeat reactive in an HIV-2 EIA and 89 (40.6%) of these were indeterminate in the HIV-2 Western blot.

Numerous investigations have tried to determine the significance of, and basis for, indeterminate results in HIV-1 Western blot tests.[19-27] Perhaps of most interest is the fact that indeterminate blot patterns are common among individuals who are nonreactive in screening tests for anti-HIV.[28] In fact, this was revealed during clinical trials, and inspection of the manufacturers' product insert shows that 15% or more of unselected, seronegative samples had indeterminate patterns. It was shown that such patterns were not associated with transmission of any infection to recipients, emphasizing both the random nature of this occurrence and the absence of a relationship to infectious disease.[28] Nevertheless, other studies have sought evidence of infection with cross-reacting viruses or have involved long-term follow-up. Other than the now well-defined circumstance in which an indeterminate pattern defines early infection, there appears to be no relationship whatsoever between HIV-1–indeterminate patterns and infection with HIV-1.[29] Furthermore, only 3 cases of true infection with HIV-2 have been found since initiation of donor testing for this virus, for an estimated rate of 1 per 28.5 million donations tested.[30] It should be noted that, in the cited case, the donor did exhibit an indeterminate pattern in 1 HIV-1 blot but was positive in the blot performed by the blood collection organization. The donor was also repeat reactive by the HIV-2 EIA. There is essentially no risk that a true HIV-2 infection would be overlooked by current algorithms.

Table 2. Anti HIV 1-2, Confirmatory Algorithm for 37.5 Million Voluntary Donations, April 1992 to July 1996

	Test Results by HIV-1 Western Blot Category (n = 46,248):		
	Positive	Negative	Indeterminate
All samples	3,799 (8.2%)	25,288 (54.7%)	17,161 (37.1%)
HIV-2 EIA RR*	2,612	194	219
HIV-2 WB Pos	828†	0	0
HIV-2 WB Ind	1,158	52	89
HIV-2 WB Neg	626	142	130

*RR, repeat reactive.

†Includes 2 true positives for HIV-2.

An immunofluorescence assay for antibodies to HIV has been approved as an alternative to the Western blot. This approach reduces, or even eliminates, the occurrence of indeterminate results.[31,32] However, it does require access to, and skills in, fluorescence microscopy. Additionally, it is a subjective procedure.

There is no clear consensus on the best approach for resolution of an indeterminate result arising during HIV screening of blood donors. In truth, given the current evaluative blot criteria, along with the routine use of the HIV-1 p24 antigen test, it is extremely unlikely that an isolated indeterminate finding has any relationship to HIV infection. Because nucleic acid testing (NAT) for HIV RNA is implemented for routine donor screening, this risk decreases still further. It has also now become clear that the evolution of seroconversion for HIV occurs over a period of days to weeks, rather than weeks to months, so that follow-up testing, if needed, should be performed after 2 to 3 weeks.[11] However, at least for the purpose of donor notification and counseling, the status of an indeterminate blot result should be readily resolved by NAT testing for HIV RNA on the index sample.

HIV ANTIGEN

Although the serological test for the HIV p24 antigen has a simple and reasonably effective immunologic blocking test for confirmatory testing, cautious regulatory standards for interpretation of this test have generated the utterly ludicrous situation in which no result can be defined as "negative." The only permissible outcomes for this confirmatory test are defined as "positive," in which there is significant inhibition when the sample is incubated with anti-p24; "indeterminate," when such inhibition cannot be detected; and "invalid" when both the inhibited and control specimens give nonreactive results.[33] In testing approximately 6.75 million donations, the American Red Cross found 1,625 repeatedly reactive results (0.024%), of which 1,491 or about 92% were indeterminate or invalid (Table 1). Among the 134 samples that gave a result that was confirmed positive on the basis of neutralization, 77 were HIV-1 RNA positive by polymerase chain reaction

(PCR), and of these, 73 were also confirmed as HIV-1 antibody positive. Fifty-seven samples did not have reproducible findings on neutralization, none was positive for HIV-1 RNA, and none showed evidence of HIV infection on the basis of repeat testing and follow-up. Thus, false-positive neutralization findings also occur. A group of 1,102 samples (comprising 794 with indeterminate, and 308 with invalid, test results for HIV-1 p24 antigen) was evaluated by PCR, and none was positive for HIV-1 RNA. It is anticipated that the interpretive criteria for this test may be modified on the basis of these data. Such donors are, however, currently eligible for reinstatement.

Again, for the purpose of donor notification and counseling, it appears appropriate to evaluate any neutralized ("positive") sample for HIV RNA, if it is anti-HIV nonreactive. The data suggest that indeterminate samples and those that give invalid results (provided sample storage requirements have been met) should not be regarded as indicative of HIV-1 infection.

HTLV

Although testing for antibodies to HTLV-I, and more recently, HTLV-II (using a combination test) have been in place for many years,[34] there is no licensed confirmatory or supplementary test available. Furthermore, investigational or "research use only" tests do not appear to be reliably available over the long term, although a variety of HTLV blots has been developed over the years. Some have been supplemented with recombinant proteins, representing an *env* glycoprotein (ie, the rp21e), or with synthetic *env* peptides that are specific for HTLV-I or HTLV-II.[35-40] There has been no set of interpretive criteria that is common to all of these procedures, although the US Public Health Service has published a recommendation that a sample be considered positive on the basis of reactivity to a native glycoprotein and to the *gag* peptide p24. This definition does not recognize rp21e as being equivalent to an *env* glycoprotein.[41] It also should be noted that some criteria recognize the presence of *gag* proteins p24 or p19, rather than p24 alone.

Experience in the American Red Cross system has been that available Western blots have generated an unacceptable proportion of indeterminate results (Table 1)—indeed, most samples give indeterminate results, and almost none can be defined as negative. Over the years, a number of more or less complex confirmatory algorithms have been developed and implemented. However, some of these have relied on tests that are no longer available or require the use of radioimmunoprecipitation, a research-level test. More recently, a system using a second licensed HTLV-I/HTLV-II EIA as the first step in a confirmatory algorithm has been developed. In this system, only samples with concordant results in the EIA tests require further testing. This approach has markedly reduced the proportion of noninterpretable results. Some 7 million donations were tested, yielding 8,661 repeatedly reactive samples by EIA. It would be anticipated that approximately 70% (or more than 6,000) of those samples would have been indeterminate if tested directly by blot. However, when those samples were tested by a second licensed EIA, only 2,989, or 35% of them, were repeatedly reactive. Of these, 2,191 (73%) were indeterminate in the blot. This approach did not, however, reduce the overall number of confirmed positive blot findings. The performance of the blot itself was not better, but the absolute number of donors with indeterminate results was reduced by 65% or more as a result of this dual EIA strategy.[42]

The original counseling recommendations for an indeterminate HTLV result were published in 1993 and have not been formally superseded. In essence, they suggest that indeterminate results are rarely associated with infections and that persistence of an indeterminate pattern for 3 months or more is almost certainly not due to infection. Testing for HTLV-I or -II DNA sequences in cellular (leukocyte) samples may be of some assistance in determining the infection status, but such testing would most likely require the collection of a new specimen.[41] Busch et al[43] evaluated blood donors who were seropositive for HTLV-I and -II and similarly found most to be indeterminate on supplemental testing. Whereas 103 of 115 blot-positive donors were positive for HTLV-I or -II by PCR (and when 10 of the 12 remaining were retested on a second occasion, 8 were positive by PCR), only 6 of 425 individuals with indeterminate results were PCR positive.[43]

HCV

Unlike the retroviruses, HCV was recognized through detection of nucleic acid sequences[44] and has not been reliably cultured in vitro. Many viral peptides have been expressed in bacteria, yeast, or

mammalian cells using recombinant DNA procedures. In addition to forming the capture reagent for EIA tests for anti-HCV, these peptides, or modifications of them, have been used to develop so-called Strip Immunoassay (SIA) tests. These tests are in many ways equivalent to a Western blot. However, instead of using an electrophoretically separated viral lysate to construct the blot, individual peptides are specifically applied to nitrocellulose paper in preestablished positions. This system permits the identification of antibody reactivity to different viral proteins. The SIA procedure has been developed in parallel (but not in synchrony) with the improved versions of the screening test. Thus, for the most part, a version 2.0 SIA was available for confirmatory testing of samples reactive in the version 2.0 EIA, and a 3.0 version of the SIA has been licensed for use with the 3.0 EIA. In the United States, there is no other licensed supplementary test for HCV antibodies, although a number of other serological tests are available outside the United States.

For the version 2.0 and 3.0 SIAs, the criterion for a positive SIA is reactivity to at least 2 viral bands at an intensity equal to, or greater than, that of the weak positive control. In addition, the band representing the superoxide dismutase (SOD) expression protein must be negative. A negative SIA has no band of greater intensity than the weak positive control. As a result, an indeterminate pattern is one with a single band with a visual intensity greater than that of the weak positive control, or a pattern with an SOD band. Again, many publications detail the significance of various band patterns.[5,6,45-49] The evaluative test of choice has been reverse transcription (RT) PCR for HCV RNA. It is clear from such studies that a minority of some (but not all) indeterminate patterns in HCV SIA are accompanied by detectable levels of HCV RNA. For example, Dow et al[5] reviewed data from 177 blood donor specimens that were reactive by EIA and positive in the version 3.0 SIA. Among 82 samples with 4 bands, 69 (84.1%) were RNA positive. Among the 54 3-band positives, 40 (74.1%) were RNA positive, whereas only 14 (34.1%) of the 2-band positives were RNA positive. Among indeterminates, the frequencies of RNA positive results were 3 of 154 for c22, 1 of 220 for c33, 1 of 191 for c100, and 0 of 380 for NS5.[5] Thus, a few indeterminate patterns may be associated with the presence of RNA, and therefore of active HCV infection. Earlier data, based on version 2.0 tests, also indicate that indeterminate samples with c22 bands are occasionally positive for HCV RNA.[48]

The overall experience of the American Red Cross in using the SIA version 3.0 (before its licensure by the FDA) involved testing 30,680 EIA repeat reactive donor samples between June 1996 and August 1998. Negative SIA results were obtained on 6,241 (20.3%) samples, 4,898 (16.0%) were indeterminate, and 19,541 (63.7%) were confirmed positive. The indeterminate group was the smallest. However, some samples that were confirmed positive also yielded equivocal results. As part of a research study linked to the use of the investigational SIA 3.0 test, samples reacting to only 2 HCV gene products were further evaluated by NAT for HCV RNA. These 2-band positives represented only 12.3% of all confirmed positives. Of the 2,347 tested, only 42% (986) were RNA positive.[50] For those 58% (1,361) with nonreactive results for RNA, donors were advised that the finding was probably due to resolved infection, fluctuating RNA levels, or a false-positive confirmatory test.

Because at least some indeterminate findings for HCV may signify infection, they cannot be completely ignored. Unlike the situation for HIV, there does not appear to be a defined progression of appearance of antibody specificities during seroconversion. Follow-up testing for HCV RNA is probably most useful in resolving an HCV-indeterminate finding, although in the absence of detectable RNA, it is probably impossible to differentiate between a pattern attributable to a resolved infection, one that represents a low level of RNA (perhaps as a result of temporal variation), or one with a nonviral cause. However, the CDC recommends that individuals with an indeterminate result in SIA be evaluated for elevated alanine transaminase (ALT) levels or by RT-PCR for HCV RNA. In the event that both results are normal (negative), no further action is recommended.[51]

OTHER TESTS

A number of other tests are routinely performed on donor samples in support of blood safety. The test for HBsAg is usually confirmed by an immunologic blocking method. This method does not generate any result formally defined as indeterminate and is not be further discussed here. The test for antibodies to hepatitis B core antigen (anti-

HBc) does not have any routinely available confirmatory or supplementary procedure. Nevertheless, a significant number of reactive results are judged to be false-positive. In addition, some samples give test signals near the cutoff, and such results may not be consistent over time. In the United States, donors are indefinitely deferred after 2 repeatedly reactive anti-HBc test results. However, the presence of anti-HBc has little clinical relevance in the absence of HBsAg. The major difficulty is indicating to a donor that he or she can no longer give blood. Somewhat similar is the issue of an elevated ALT level. In some circumstances, blood donors are still tested for ALT levels and may be deferred on the basis of their enzyme level. Again, results may be variable over time, and there is no confirmatory mechanism. Finally, there are a number of different algorithms for syphilis testing. Again, there are no results that can be formally defined as indeterminate, but there are patterns of results that are not readily interpretable.

ISSUES ASSOCIATED WITH INDETERMINATE RESULTS

The major objective of confirmatory or supplementary testing is to improve the accuracy and significance of information derived from routine blood donor screening tests. A result of "indeterminate" clearly does not fulfill this bioethical imperative. Yet the regulatory posture in the United States requires that the interpretation in the manufacturers' product insert must be provided to the donor. As pointed out previously, an indeterminate interpretation occurs as frequently, or more often than, a negative one, and in a number of cases, more frequently than one that is positive. Furthermore, although there is guidance (or regulation) about the interpretation of a positive test result, there is no formal guidance about the best way to manage indeterminate results. Such guidance as is available appears to be out of date (see, for example, the CDC recommendations on HIV-1 or HTLV Western blot interpretation).[10,12,41] It is hard to see how this situation can be allowed to continue.

The regulatory status of supplementary or confirmatory tests for blood donor screening in the United States contributes significantly to this problem. First, licensure is required for approval of confirmatory tests, and the conditions for such licensure are essentially the same as those for the primary screening test. Obtaining licensure is a lengthy and expensive process and, given the relatively small market, is understandably not attractive to manufacturers. Once a test is licensed, there is very little incentive to improve or replace it. The barriers to entry are so great that, at this time, it does not appear as though any manufacturer is prepared to develop a licensed supplementary test for HTLV-I or -II. It is curious and inexplicable that, despite these regulatory requirements, the tests in current use (including those licensed by the FDA) generate such high proportions of indeterminate results.

Experience has shown that confirmatory testing is likely to undergo the same level of regulatory scrutiny as any other phase of preparation of products for transfusion. In part, this seems to be attributable to the nature of the regulatory process itself. This makes only limited sense if the purpose of confirmatory testing is to support donor notification and counseling. Additionally, however, confirmatory testing has become a formal component of so-called reentry algorithms. In these circumstances, from a regulatory perspective, the confirmatory test might have a regulatory impact on the safety and purity of blood components. This is because a donor would be accepted for reentry on the basis of a negative confirmatory test. Any error in that testing, once recognized, thus may lead to recall of future products (including finished plasma derivatives) from the subject donors. This is more than a theoretical concern: numerous laboratories have been the subject of vigorous regulatory action on the basis of their confirmatory testing, and consequently, there have been multimillion dollar recalls of plasma derivatives. These stringent requirements are a heavy burden to place on complex, research-level tests that are almost invariably evaluated subjectively.

As pointed out, the occurrence of many indeterminate patterns is essentially random and is not associated with any identifiable cause. As a result, the number of indeterminate findings is proportional to the number of samples tested rather than to the prevalence of infection. As a consequence, use of a primary screening test with poor specificity will ultimately lead to a greater proportion of donors being classified as indeterminate. This is amply illustrated by the data developed from the dual EIA strategy for HTLV confirmation.

The confirmatory algorithms in use today derive almost directly from the technology of the early 1980s. At that time, serologic assays were the norm. Understandable and justifiable desire for a confirmatory test for HIV antibody resulted in the adoption of a technology that was far from appropriate at the time. This perhaps blinded us to other approaches, even to the extent that the SIA was developed as a form of artificial blot. Attempts to develop objective confirmatory procedures (such as multiwell enzyme-linked immunosorbent assays) were met with little enthusiasm. The need to provide accurate information to blood donors has rightly continued to be a priority. However, the general approach to dealing with the difficulties of the indeterminate result has been to add other tests, in effect developing a confirmatory test for the confirmatory test itself. Additionally, it has now become apparent that blots (particularly those for HIV and for HTLV) can generate false-positive results. Perhaps it is time to rethink the entire approach, starting with a reconsideration of the need for Western blots or similar procedures. As has been amply illustrated above, these procedures create uncertainty rather than precision and compromise the integrity of our duty to the blood donor.[52]

CONSIDERATIONS FOR THE FUTURE

The primary focus of confirmatory testing should be provision of accurate clinical information for the donor. This should be separated from regulatory requirements for reentry of donors with false-positive screening test results. Ideally, confirmatory protocols should give unequivocal answers, should be based on objective tests, and should be evidence-based. Two key priorities for counseling are: (a) to provide effective advice to infected individuals so that they can take care of their health and reduce the risk of infecting others; and (b) to reassure those with false-positive screening tests that, although they may no longer give blood, they are not at increased risk of developing disease. A move in the right direction might be to reevaluate interpretive criteria and classify irrelevant blot patterns as negative. However, it is perhaps time to step back and reconsider the whole process.

It is understandable that regulatory agencies should be cautious about testing that leads to donor reentry or reinstatement. Licensed tests, capable of detecting minimal or partial antibody responses to infection may be most appropriate for this purpose. Thus, it would perhaps be appropriate to use 2 different algorithms: a simple, unequivocal approach for donor notification and counseling and a more rigorous procedure for donor reentry, should it prove necessary.

The simplest approach recommended for confirmation is to retest an EIA-reactive sample with a different, licensed EIA procedure. A sample that is reactive in only 1 EIA test is most likely to be false-reactive and should not require any further testing. Clearly, there are circumstances in which such a sample could be a true positive as a result of sensitivity differences between tests, but this is extremely uncommon. Studies on HTLV antibody tests have validated this approach. Samples that are reactive in 2 different EIA tests have a higher probability of being truly positive than those that are reactive in a single test. Those samples could be further evaluated by use of sensitive genome amplification tests. Such testing would be expected to accurately identify all individuals who are seropositive for, and infected with, HIV, for example. This may not be true for HCV, however, because individuals with detectable antibody responses to HCV may demonstrate fluctuating levels of HCV RNA. The use of ALT testing may be helpful. It should be noted that an algorithm for HCV screening, published by the CDC, includes the option of testing an EIA-reactive sample for RNA and reflexing only the negatives to the SIA.[51] A final specific difficulty with this general approach is the need to look for cell-associated DNA to confirm HTLV infection (although there have been publications that indicate that HTLV genome sequences can be detected in plasma).

COMMENT

The concept of confirmatory testing in support of EIA screening tests is admirable. However, in the context of transfusion medicine, at least in the United States, the practice has been less than optimal. For HIV, HTLV, and HCV, the most commonly used format for confirmatory testing generates an unacceptable number of outcomes that cannot be interpreted. Furthermore, regulatory imperatives have given few options for change and tend to inhibit development of alternate approaches. Attempts to use available technologies in support of both counseling and blood donor reentry

seem to have confounded the issue. Emerging technologies (particularly nucleic acid testing) offer new and more meaningful approaches to defining the significance of serological test results. It would appear that the purposes of confirmatory testing for donor notification and counseling could best be served by the use of a second EIA and an appropriate nucleic acid test.

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