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The Effects of the AIDS Epidemic on the Safety of the Nation's Blood Supply

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Synopsis

Present safeguards for the blood supply consist of three tiers of protection: donor deferral based on a donor's history of risk factors, confidential exclusion of blood units from donors with self-admitted risk factors, and testing of the blood itself.

Before the discovery of the AIDS virus in 1983 and 1984, there was no specific test relevant to AIDS that could be used to help improve the safety of the blood supply. The first step was intensified efforts, based on what was then known of the epidemiology of the disease, to take donor histories to identify risk factors. The first specific tests were for the detection of antibodies to the virus and came into use in 1985.

The general features of AIDS are described, together with the scientific rationale for the various types of laboratory tests, those for the virus itself, antigens, antibodies, the genetic material of the virus, and T4 lymphocytes. General characteristics of the tests are reviewed.

Since testing began, about 30 million units each of blood and plasma have been screened. More than 3,000 infected persons in the blood donor group have been identified as HIV-antibody positive. Thirteen cases of transfusion-associated infection have been documented. They are believed to have occurred because a detectable level of antibodies had not yet formed in the infected donors. Currently, such transmission is thought to occur once in about 40,000 to 250,000 donations, a dramatic improvement from 1983.

WITHIN A YEAR after acquired immunodeficiency syndrome (AIDS) was identified as a new clinical condition in 1981, accumulating evidence showed that the infectious agent could be transmitted from an infected blood donor to a transfusion recipient through the transfusion of blood, or certain products derived from blood, such as red cells, platelets, and antihemophilic factors, notably Factor VIII (*1*).

Because the AIDS virus had not been identified, there was no possibility at that time of a specific test to identify infected blood. However, in 1983, steps were taken to improve the situation by educating potential donors about AIDS and by asking persons who might be at risk for infection not to donate.

An indirect screening procedure was put in place in response to growing awareness of the need to protect the blood supply. Based upon what was then known of the epidemiology of the disease, the Food and Drug Administration (FDA) and the blood and plasma collecting organizations agreed to intensify and expand efforts to screen prospective donors by taking detailed personal histories.

The histories were designed to identify an individual donor's risk factors and to use the risk factors as criteria for voluntary donor exclusion.

Methods of voluntary deferral of donors have improved with time. The educational information about risk factors now focuses on the identification of risk behavior, rather than on the question of whether one belongs to a specific risk group. It is now possible for donors to indicate confidentially that their blood unit should be used only for laboratory purposes and not for transfusion. This enables blood collection centers to exclude from clinical use those blood units whose donors have identified risk factors.

The discovery in 1983 of the virus causing AIDS, now called the human immunodeficiency virus (HIV), opened the way to development of specific tests directly relevant to the disease, although the technology for test development, the ability to grow the virus in the laboratory and to purify it, did not emerge for another year.

The first specific AIDS-related tests which became feasible were for the detection of antibodies to the virus. In 1985, using well-established technologies, several groups in western Europe and North America developed antibody tests. The tests were applied rapidly to screening blood and plasma donations as a public health measure. Much of what was learned in that experience continues to be valuable in evaluating the acceptability of new tests as they become available.

Scientific Rationale for Tests

The following is a simplified description of the general features of the disease and the biological events which occur after infection with HIV. The stages of the development of the infection are shown in figure 1. They are described to point out the scientific rationale for the various types of laboratory tests which have been considered relevant. Figure 2 shows the sequence of events following infection based on what is currently known about the biology of the syndrome. Specific features are pointed out as having direct relevance to the potential usefulness of various tests for the detection of the infection.

Stage 1. HIV enters the body, attaches to cells, reproduces itself, and infects an increasing number of other cells. In the reproductive process, the virus turns its genetic material into DNA by the use of an enzyme called reverse transcriptase and then integrates its DNA into the chromosomes of the infected cells.

Figure 1. Model of active and latent cell infection by the human immunodeficiency virus

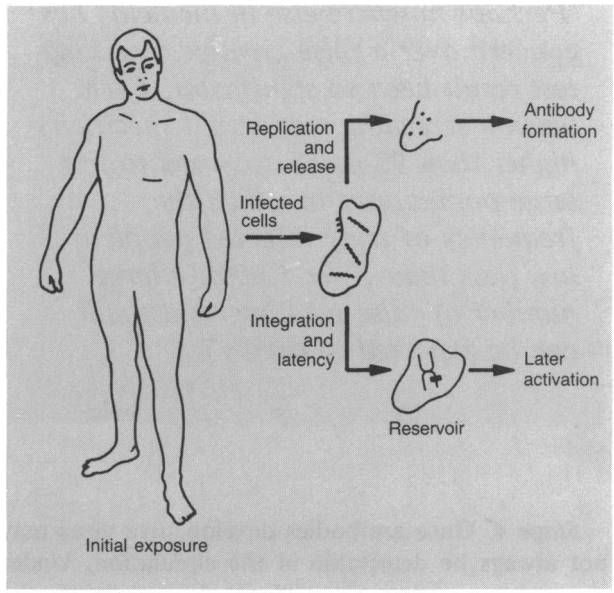
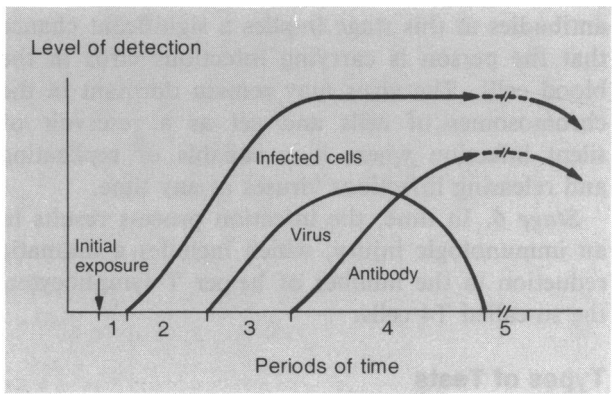


Figure 2. Schematic diagram of the sequence of biological response to infection with HIV



Stage 2. Some of the infected cells synthesize large amounts of new viruses and release them into the plasma, resulting in a viremic state. In time, a sufficient number of viruses will be produced to permit their detection.

Stage 3. Viruses interact with the immune system, which, in response to these antigens, or foreign molecules, produces antibodies to various viral proteins. An antibody test to detect an infected person is ineffective until antibodies are present. The time needed for a person to respond immunologically is generally 6 to 12 weeks from the time of infection, but unusually long periods of 6 to 30 months have been described.

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Stage 4. Once antibodies develop, live virus may not always be detectable in the circulation. Under these circumstances the evidence for being infected comes from the presence of antibodies and the finding of blood cells with integrated viruses.

Stage 5. The virus and the antibody coexist in a phase which may last for years. The detection of antibodies at this stage implies a significant chance that the person is carrying infectious virus in the blood cells. The virus may remain dormant in the chromosomes of cells and act as a reservoir of silent infection where it is capable of replicating and releasing infectious viruses at any time.

Stage 6. In time, the infection process results in an immunologic injury, which includes a dramatic reduction in the number of helper T-lymphocytes, the so-called T4 cells.

Types of Tests

This very simplified picture of the biological events which occur after infection with HIV shows that, in principle, it is possible to have at least six different types of AIDS-related tests.

Virus testing. Laboratory evidence for the presence of the virus itself can be obtained by testing for one or more of the proteins of the virus, or by trying to grow the virus *in vitro* in a cell culture system. At present, commercial tests for viral proteins are under development, but their usefulness in screening blood donations has not been fully established. Tests for infectious virus are feasible, but they are fairly complex and require specialized facilities and training, making them unsuitable for screening purposes.

Antibody testing. With relatively few exceptions, antibodies are formed and persist after a person is exposed to an infectious agent. Antibody tests provide a means of identifying a person who has been infected with the virus at some time in the past. Antibody tests do not, however, give direct information about when the person was infected or whether that person has live HIV in plasma and other body fluids.

HIV antibody tests in use, including unlicensed research tests, are the enzyme-linked immunosorbant assay (ELISA), also a test for the antigen; hemagglutination inhibition (HI); the Western blot (WB); immunofluorescence (IF); radioimmunoprecipitation (RIP); and particle agglutination (PA).

Reverse transcriptase testing. An alternate approach to testing for evidence of the virus itself is to assay for the activity of its unique enzyme, reverse transcriptase (RT), which is present in each viral particle. A test for this enzyme could serve as a biological marker for the virus, but data show that this is not as sensitive as other tests that can be done on blood samples.

Nucleic acid testing. The virus can be present in a subtle or cryptic form as nucleic acid integrated into the chromosomes of some cells. In this case, it is possible to identify infected cells by testing a sample for the presence of viral nucleic acid sequences by using hybridization techniques, including a new technique, amplification by polymerase chain reaction.

T4 lymphocyte number testing. The virus preferentially attacks a certain subset of lymphocytes, the T4 cells. Taking advantage of that fact, the number of T4 cells in an infected person, as determined by immunofluorescence techniques, is a direct indicator of the degree to which the virus has impaired the immune system. The exact mechanism of cell destruction has not been determined.

Test Characteristics

The appropriate type of test for a clinical diagnostic or screening purpose depends, first, on the nature of the test in relation to the biology of the infection and, second, on the specific question that one is interested in answering. Secondary considerations must be taken into account, such as cost and technical performance characteristics.

Characteristics and uses of AIDS-related tests

Test	Technical complexity	Sensitivity	Specificity	Uses
Virus: cell culture	High	Medium	High	Clinical diagnosis, research
Antigen: ELISA	Medium	Medium	High	Clinical diagnosis, research
Antibody:				
ELISA	Medium	High	High	Screening, clinical diagnosis, research
HI and PA	Low	High	Medium	Screening, clinical diagnosis, research
IF	Medium	High	High	Screening, clinical diagnosis, research
WB	Medium	High	High	Clinical diagnosis, research
RIP	High	High	High	Research
Reverse transcriptase: enzyme	High	Low	High	Clinical diagnosis, research
Nucleic acid: hybridization	High	Medium	High	Research
T4 number: cell count	Medium	Low	High	Clinical diagnosis, research

NOTE: ELISA is enzyme-linked immunosorbent assay; HI is hemagglutination inhibition; PA is particle agglutination; IF is immunofluorescence; WB is Western blot; and RIP is radioimmunoprecipitation.

The table shows, for each of the types of test, some general characteristics which are directly relevant to whether a test is reasonable to consider in various settings. A major consideration is the technical complexity of the test in relation to the question being asked and the setting in which it is to be used. A technically difficult test may be reasonable for research purposes in settings where all of the scientific infrastructure is in place, and be inappropriate for screening purposes even in a technologically advanced country.

Sensitivity and specificity of the tests are important considerations. Ideally, the test would be the most sensitive possible and 100 percent specific. The perfect test would be able to detect extremely low levels of antibody, antigen, or virus without failing to recognize an infected sample (being false negative), and without responding positively to an uninfected sample (being false positive). No biological system is perfect, however, and there are always issues related to how good a test really is in terms of its sensitivity and specificity.

To answer those questions, tests must be evaluated in a number of ways by different groups in different settings. Because of the intense international interest in AIDS, all of the commercial antibody tests which have been developed to date have been assessed in many laboratories throughout the world, and a large number of reports on them have appeared in the medical literature. The result has been a steady refinement and improvement in AIDS-related antibody tests since they were first introduced commercially in 1985.

The acceptability of a given test system depends on two levels of what might be called quality control data. The first is the general body of data which must be developed to show that the test performs to expectations in the target populations in which it is to be used. Those data are used to qualify the test system and to establish that, at least on the basis of a limited amount of information, it appears to perform in the expected manner. The second level of control, imposed on each set of tests as they are run in a laboratory, is to determine whether the test is performing at the level expected, according to its technical specifications. For that purpose, the test must have appropriate positive and negative control samples integrated into its configuration to show that it is behaving as required on each individual run and that nothing has gone wrong.

Assuming that a test has acceptable technical characteristics and that the quality control aspects have been addressed, there remains the issue of interpreting and acting upon the data developed during the use of the test. Perhaps nowhere else in medicine has concern over a false-positive screening test result been so significant. Even when a screening test with a specificity higher than 99 percent is used to test large populations in which the frequency of truly infected people is low (less than 1 per 1,000), a large number of false-positive results still can be expected to occur.

In order to distinguish the true positives from the false, it is necessary to check the positive samples with a highly specific confirmatory test based on independent scientific principles. The most frequently used confirmatory test has been the immunoblot (also called the Western blot), but indirect immunofluorescence and radioim-

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munoprecipitation have been used successfully. Each carries its own set of technical advantages as well as problems; the results of each must be interpreted by skilled and experienced personnel. The approval by FDA of the Western blot test in 1987 has allowed a large number of donors who were deferred on the basis of a false-positive test to be reinstated (*2*).

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Transfusion Infections

Unfortunately, like any other biological system, the screening test is not perfect, and 13 documented cases of transfusion-associated infections have occurred (*4*). These cases happened because antibody had not yet formed in the infected donors when the screening was performed and the results of the screening test therefore were falsely negative.

How often does this happen? Because it is a rare event, and because there is no routine followup possible for all donors and all recipients, it is difficult to give a precise number. Based on what is known currently, it probably occurs in between 1 in 40,000 and 1 in 250,000 donations (*4,5*). It is encouraging to note that the percent of antibody-positive donations has decreased from .04 to .01 since the beginning of testing.

The problem of false-negative screening tests, while essentially a technical problem, reflects the larger issue of new AIDS infections. False-negative screening test results are associated primarily with newly infected persons because the period before antibodies may be detected is usually 6 to 12 weeks after infection. However, as information, education, and other prevention activities become more effective, the false-negative problem could essentially be eliminated as the pool of newly infected persons is reduced together with the number of newly infected persons who choose to donate blood. Until then, we will be seeing a few infected units slip through the screening process. However, like virtually every other aspect of AIDS, there are regional and local differences to the problem that reflect the geographic distribution of people infected with HIV.

Antihemophilic Factors

Transmission of AIDS by antihemophilic factors has remained a significant concern even with the addition of antibody screening because of the fact that these products are made from pools of plasma from very large numbers of donations, 20,000 or more in each lot, so that the risk of an infectious pool remains significant. A remarkable reduction in the infectivity of these materials was accomplished empirically in 1984 by adding a heating process to inactivate the virus. Nevertheless, complete elimination of the AIDS virus has been difficult to achieve for these products, and there have been many additional efforts to develop better inactivation methods. Most recently, novel techniques such as pasteurization in the liquid state, purification using monoclonal antibodies, and extraction with solvents and detergents have offered the prospect of increased safety (*6*).

Current Problems and Progress

What has resulted from the past 2 years of testing blood and plasma donors? We have made blood and blood products very much safer than they were. Further, we have learned that testing

for infection with HIV is unlike any other test in medicine because of the intense public concern over the results of testing, and the technical aspects of screening that complicate its use.

There is the problem of false-positive tests. By setting the sensitivity of the tests so high that we are virtually certain of capturing in the screening net all truly antibody-positive persons, we have to pay the price of capturing some noninfected persons.

There is the problem of sorting out the true positives in a second round of testing, using a different method that provides greater specificity. And, there is the issue of how to handle the positive test result information, both from the point of view of notifying the person and of maintaining confidentiality (*7*).

It is to the credit of the blood and plasma collecting organizations in this country that they undertook these challenges together with the Federal Government in a cooperative and efficient way in a highly charged atmosphere, and made the system work. The task of perfecting screening and diagnostic tests for AIDS is far from over. The public and the scientific community continue to seek the elusive goal of risk-free transfusions and blood products. The challenge remains to develop forms of testing which can detect the AIDS virus prior to the development of antibodies.

A new dimension to the problem is the recognition of a second form of the AIDS virus, human immunodeficiency virus-2 (HIV-2), that may escape detection by the current screening tests. The first case in the United States of AIDS associated with this virus was described recently in a person from West Africa, where HIV-2 is endemic (*8*). Surveillance for infection by this virus has been intensified in order to give early consideration to the routine use of specific screening tests for HIV-2. Fortunately, such tests are now being evaluated and should be available if the need for routine use becomes clear.

The present three-tiered system of safeguards, donor deferral based on a history of risk factors, confidential exclusion of donors with self-admitted risk factors, and testing of the blood itself, has dramatically improved the safety of blood and blood products since 1983. There is reason to believe that continued technological and social advances will result in even greater safety. There were start-up problems with blood testing, but overall, it is remarkable how rapidly and successfully we have moved into an extremely difficult, sensitive, and unexplored area.

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