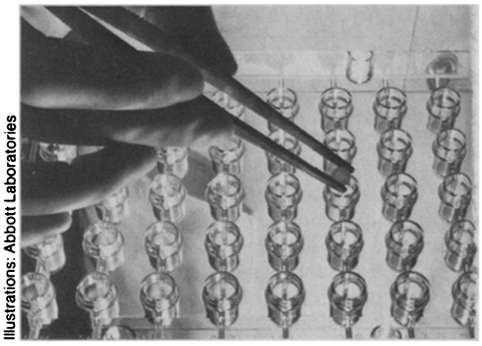
AIDS Blood Screens: Chapters 2 and 3



Illustrations: Abbott Laboratories

The screening method guarding the nation's blood supply from the AIDS virus is good but not perfect; new, soon-to-be-approved procedures will solve some but not all of the problems

By JOANNE SILBERNER

In March 1985, the U.S. Food and Drug Administration approved the marketing of several blood screens that detect antibodies to the AIDS virus. The nation's blood banks immediately began screening donated blood and pulling positive units from their shelves. The screens have been credited with halting new infections from blood or blood products.

But while the tests are very sensitive — they identify just about all contaminated blood — they also have their problems:

• They give positive results for some blood that would not transmit AIDS. In fact, says Max Essex, an AIDS researcher at Harvard University, "Ninety to 95 percent of the people who test positive don't really have the virus." Included in this group are people who test positive because they have other cross-reactive antibodies, unrelated to the AIDS virus.

• Of the apparently healthy "true positives" it does identify, the type of screen used today does not pinpoint which people will go on to develop AIDS within five years — a fate that will befall an estimated 20 to 30 percent of them, according to the Centers for Disease Control in Atlanta.

• A small number of people whose tests come up negative actually have AIDS virus in their blood, and at least one person has contracted the virus from an "antibody-negative" donor.

In the wake of such difficulties, researchers and industry have been searching for a second-generation blood screen, and several are expected to be approved by the Food and Drug Administration (FDA) in the next few months. While the second-generation tests, like the first generation, detect antibodies rather than the virus itself, they are more specific and less prone to false positives.

Unfortunately, they won't be any better than the first-generation tests at picking out people who harbor the virus but have not raised antibodies against it. To detect these antibody-negative, virus-positive people will take third-generation tests that hunt for the virus itself. Such tests are already being developed by several companies.

The blood screens are the first practical application of AIDS laboratory research. Since there is as yet no cure for AIDS, screening blood, sticking to safe sexual practices and avoiding intravenous drug abuse are the only steps that can be taken against the syndrome.

The American Red Cross, while following the development of the second- and third-generation screens, is expressing confidence in the sensitivity of the current antibody screen despite its problems. "Since the initiation of the screen there has not been a report of any transfusion-associated AIDS," says Joseph O'Malley, a medical specialist at the American Red Cross in Washington, D.C., "although recently there has been one case of seroconversion [development of antibodies]."

The seroconversion, detailed in the June 20 MORBIDITY AND MORTALITY WEEKLY REPORT, occurred in a 60-year-old man who had received blood during surgery in August 1985. The blood, which had tested negative, came from a donor who had had homosexual relations with one partner; a blood sample taken several months after the donation that caused the seroconversion tested positive. "The blood was [initially] nonreactive due to the fact that the donor was in a 'window' period between infection and seroconversion," says O'Malley. "As a homosexual, the individual should not have donated blood. The only way around a case like this is to develop a test for the virus itself."

Both the first- and second-generation screens are ELISAs, enzyme-linked immunosorbent assays. They are augmenting the crude screening — simply requesting that members of high-risk groups not donate blood — on which blood banks had been depending.

In an ELISA, the blood is added to small wells containing bits of the AIDS virus, which was previously known as HTLV-III or LAV-I, but has been recently designated HIV (human immunodeficiency virus) by an international committee of virologists. If there are HIV antibodies in the blood, they'll stick to the virus. To detect such antibodies, a second, enzyme-linked antibody is added, which will attach to the antibody-virus complex, if present. When a chemical with which the enzyme reacts is added, the enzyme itself changes color, signaling the presence of the antibody.

The problem with the first-generation screen, which costs about $4 per blood sample, is that nonspecific cellular debris from the initial cell culture in which the virus was grown can be present in the well with the virus. Some people test positive not because they have antibodies to the virus but because they have

AIDS consensus: More research

The National Institutes of Health in Bethesda, Md., convened a panel of experts earlier this month to hear reports on the currently used screen and to evaluate AIDS-antibody testing. They concluded that the screening represents "remarkable progress" in applying basic research, but that the current technology does have its flaws.

While total elimination of infected blood from the national supply is not "immediately feasible," it remains a desirable goal, they said. Among their conclusions:

• People anticipating surgery and able to pre-bank their own blood should do so, since it "is the safest form of transfusion therapy." Blood banks, they suggest, should make the option available and inform physicians and patients about its advantages.

• Research is needed to develop more sensitive antibody tests and easy-to-do confirmatory tests. Ultimately, new methods of detecting virus and virus-specific proteins should be developed.

• The practice of allowing ELISA-positive, western-blot-negative donors to continue to donate blood that will be discarded is unfair to the donors, who should be informed of their status.

"The primary means of preventing AIDS and protecting the public health is through the responsible behavior of persons," the 13-member panel said in its consensus statement. "Every aspect of the problem," the panel concluded, "requires continuing research."

— J. Silberner

antibodies to the cell in which it was grown. Included in this group are monogamous women who, in the course of bearing several children, were exposed to foreign white blood cells and developed antibodies to them.

The American Red Cross, which collects and distributes half the blood donations in the United States, rechecks initial positives with two more ELISAs. If either is positive, the blood is considered a repeat reactive. About 1 percent of Red Cross donors are initially reactive; about 0.30 to 0.35 percent—30 to 35 percent of initial reactives — are repeaters.

Repeat reactive blood is tested with what is called a western blot, or immunoblot, assay. In this procedure, which costs about $65, suspect blood is added to blotting paper that contains AIDS virus proteins of different sizes. Antibodies specific to the particular proteins will stick. As with ELISA, an enzyme-linked antibody is added; in this case, when a chemical with which the enzyme reacts is added, the complex turns color and can be detected visually.

About 0.025 percent of the Red Cross donors — roughly 8 percent of the repeat reactives on the ELISA test — wind up with positive western blot tests. The Centers for Disease Control estimates that 1 million to 1.5 million apparently healthy individuals in the United States are antibody positive and thus presumably western blot positive.

The Red Cross notifies donors who are western blot positive but not those who are western blot negative, even if they are repeat reactives. To safeguard the blood supply, the Red Cross is discarding blood from people who are repeatedly reactive on ELISA but tested negative on western blots. "We're throwing out a lot of blood," O'Malley says.

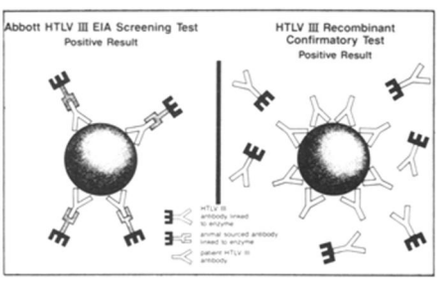
A variation on the current ELISAs received FDA approval in February. Made by Genetic Systems Corp. of Seattle, the test is more a cousin than a second-generation descendant: It is an ELISA that uses a viral isolate provided by the Paris-based Pasteur Institute and grown in a different cell line that reduces the number of false positives caused by reactivity against non-AIDS proteins.

The antibody screens produced by other U.S. companies are based on a virus and cell line developed at the National Institutes of Health (NIH) in Bethesda, Md., and patented by the U.S. government. That patent is currently being contested by the Pasteur Institute, which claims that NIH researchers depended on viral isolates the French group had shared with them, and that Pasteur's earlier patent application should be the valid one. The U.S. patent office three months ago put the onus on NIH to prove that its antibody detection method predates Pasteur's.

The primacy question could become moot when screens that use proteins produced by genetic engineering rather than by HIV itself are approved. The benefit, notes Hubert Schoemaker, president of Centocor, a biotechnology company, is that these screens don't use the cell-grown virus. This eliminates the cellular debris that causes nonspecific reactions, so that a positive reading will reflect true HIV antibodies. In addition, he notes, genetically engineered proteins eliminate the hazard of working with live virus. Centocor, based in Malvern, Pa., has a recombinant protein product Schoemaker believes is near FDA approval.

But the second-generation antibody tests won't take care of everything. False negatives, says Harvard's Essex, are "still a limitation in the first- and second-generation test. Some percentage of people infected with the virus — the best figure used is 5 percent, but nobody knows exactly — don't have detectable antibody."

Included in the antibody-negative, virus-positive group are people who picked up the virus only recently and haven't yet produced antibodies. It can take weeks or months following infection for antibodies to appear in the blood.



In a positive ELISA (left), the patient's antibody sticks to AIDS protein coating beads (also in photo, top) and is detected with an antibody to human antibodies. As confirmation (right), the patient's antibodies displace enzyme-linked antibodies.

Jay Levy of the University of California at San Francisco has used immunofluorescence to find antibody-negative, virus-positive blood. In the procedure, he treats potentially infected cells with a chemical that opens them up, allowing viral antibodies to enter. These antibodies, in turn, can be identified by fluorescent tags. "We have seen [in the same blood sample] a positive by immunofluorescence and negative by ELISA and immunoblot," says Levy. "I think it's rare, but it does occur."

The only way to find antibody-negative, virus-positive samples is by checking for the virus directly instead of the antibody footprints. This is the goal of the third-generation tests. Detecting virus is difficult because HIV generally is present in only low concentrations.

At the moment, such testing is impractical. "The only sure way of showing it [the virus] is there is to grow it out," says O'Malley. "But that's extremely difficult and expensive." The virus has to be grown in cell cultures kept alive while it replicates. "Even some of the largest medical research groups in the country have been tripped up trying to isolate the virus," says O'Malley.

In one type of third-generation test, DNA probes use one side of the virus's double helix to seek out its complementary half. L. R. Overby of Chiron Research Laboratories in Emeryville, Calif., says, "There's no evidence even with [easy-to-use] probe technology that there's sufficient virus to be detected that way." The company has been working on a probe, but it is being designed as a research and clinical tool, not as a simple screen. It may prove useful for determining whether a person with symptoms of AIDS actually has HIV, says Overby.

Centocor is also working on a method to detect AIDS virus. Theirs is not a DNA probe but will depend on antibodies that bind to the virus's genetic material. Such direct testing, however, won't necessarily be more practical than antibody tests for screening blood, he believes.

Cetus Corp. of Emeryville, Calif., is going for a DNA probe in a novel manner. Because the virus's concentration in the blood is so low, they have developed a series of chemical steps that will reproduce any HIV present in a blood sample. A subsequent DNA probe will have a greater amount of HIV to survey and, therefore, a much greater chance of determining whether the blood is infected.

Researchers from the University of California at San Francisco are working on another way to test for the virus. At the International Conference on AIDS last month in Paris, Jacques Homsy described a test for an AIDS virus protein that involves pitting suspect blood against known levels of recombinantly produced protein and measuring its ability to bind to antibody that is specific to the protein.

If the blood being tested contains that particular virus-bound protein, the protein will compete with its recombinant twin, and less of the recombinant protein will bind to the antibody. Conversely, if all the recombinant protein is bound, it means no virus is present. The test, Homsy claims, yields few false positives and can detect as few as 100 infected cells in a blood sample.

Tests for virus are expected to eliminate the handful of exceptions that slip through the current screening process. "If you include screening for risk groups and pick out antibody positives, you get the majority of the dangerous [blood] units out," says Thomas Merigan, a specialist in infectious diseases at Stanford University. "It would be nice to have them all out." □