Improving the AIDS Test

Genetic engineers offer a new approach to AIDS-antibody testing

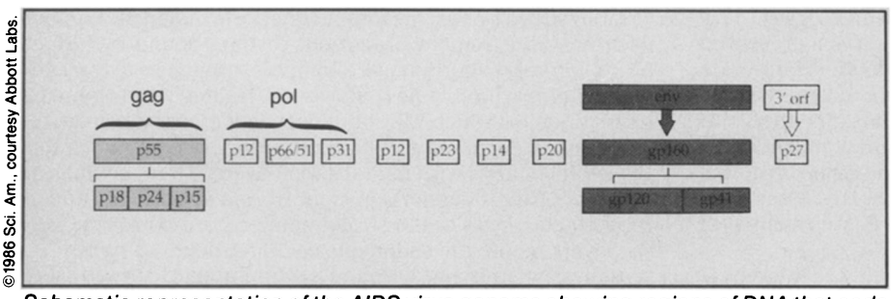
By RICK WEISS

Only three years after the first AIDS antibody test was approved by the Food and Drug Administration (FDA) for screening donated blood, a promising new generation of AIDS tests is on the horizon. The new tests, some of which may be approved later this year, use recombinant DNA technology that didn't even exist a few years ago. They reflect a changing biomedical and political climate in which AIDS testing is increasingly being performed for purposes other than blood donor screening, making it more important than ever to minimize false positive or ambiguous results.

Ongoing trials with several varieties of second-generation AIDS tests — which use either synthetic proteins or proteins made by genetically engineered microorganisms, instead of proteins from real AIDS viruses — have for the most part shown that the newer tests are at least as sensitive and specific as current tests. According to scientists and public health officials who gathered for a workshop last month at the National Institutes of Health in Bethesda, Md., the new tests offer a number of potential advantages as well.

In many cases the newer tests are able to detect AIDS antibodies in blood at earlier stages of infection than can the currently approved tests. Moreover, some require no sophisticated equipment or refrigeration. Thus, for the first time, screening of donor blood may become practical in developing countries. Several of these countries have a much higher prevalence of AIDS than does the United States and are experiencing high rates of infection via hospital transfusions of unscreened, contaminated blood.

"There is a real need for improvement in testing," says Michael O'Shaughnessy of the Canadian Federal Center for AIDS. He says that with the relatively low but persistent number of false positive results on initial screening and a frustratingly high number of indeterminate results on confirmatory tests, "it's my opinion that recombinant antigen tests will sooner rather than later replace the current [tests]."



Schematic representation of the AIDS virus genome showing regions of DNA that code for production of, among other things, viral core protein (gag), reverse transcriptase (pol) and envelope proteins (env). Most second-generation AIDS tests use synthetic or genetically engineered peptides from the gp 120 and gp 40 regions of the env sequence, in part because the genetic code in that area tends to be highly conserved, or constant, among different strains of HIV.

The first test to detect antibodies to the AIDS-causing virus, HIV, was licensed by the FDA in March 1985. Seven other of these so-called ELISA tests have been approved since then. All of them use protein fragments, or peptides, from cultured AIDS viruses, which bind to and label AIDS antibodies in sampled blood. The Western blot confirmatory test — the only FDA-approved test for confirming ELISA results — was licensed for commercial use in 1987.

Today, among the 17 or so other AIDS diagnostic products in various stages of development are a number that use recombinant DNA-produced or laboratory-synthesized peptides that mimic key peptides in the AIDS virus, according to the Washington, D.C.-based Pharmaceutical Manufacturers Association.

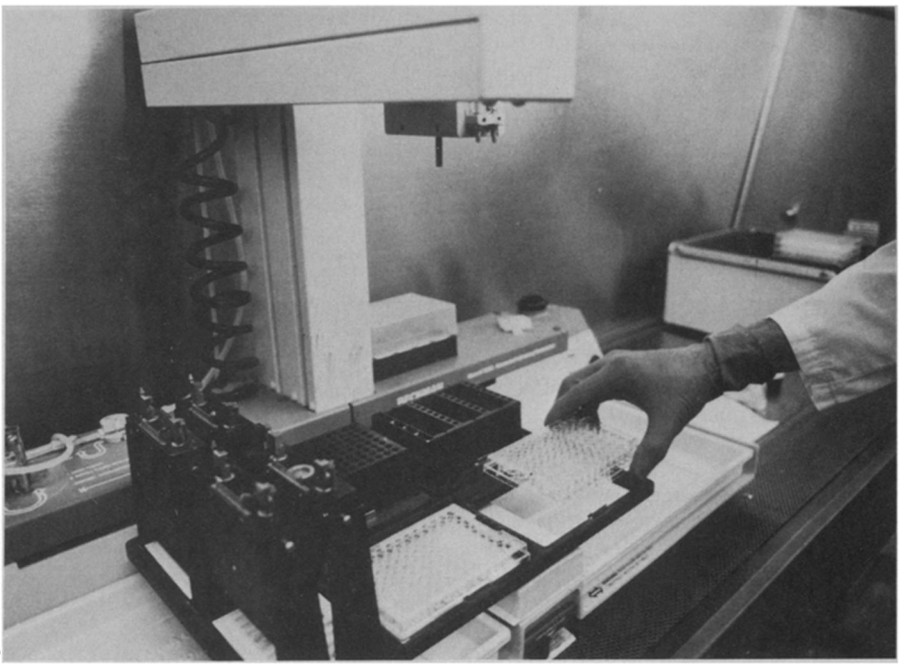
Like the current ELISA tests, the new tests provide antigenic material for AIDS antibodies to bind to. In most of the new tests, these bound antibodies are then exposed to labeled "anti-antibodies." If there are no AIDS antibodies to bind to, the anti-antibodies wash away. Conversely, continued presence of the label (generally a color reaction or fluorescence) indicates a "positive" finding of AIDS antibodies.

Several of the largest pharmaceutical and diagnostic companies in the United States, including SmithKline Bioscience Labs, DuPont and Abbott, as well as a number of smaller biotechnology companies, are developing such tests. According to the FDA, which doesn't give details about pending applications for test licensures, "a few" companies have already filed for approval of their new tests. Others are gathering data from clinical trials both domestically and abroad. At least three of the tests are already approved for use in some European countries.

In addition, Cambridge Bioscience, a biotechnology company based in Worcester, Mass., has developed a five-minute "slide agglutinin" test that uses microscopic latex beads coated with genetically engineered AIDS-virus antigens. In the presence of AIDS antibody, the latex beads clump together when a bead-bridging "anti-antibody" is added. The reaction can be read visually under a bright light, eliminating the need for sophisticated equipment. The test has been used successfully in blood screening programs in Africa, and approval is pending in the United States.

The advantages of using laboratory-derived peptides are numerous, researchers say. Perhaps most important, mass-produced synthetic peptides can be highly purified in the laboratory, virtually eliminating extraneous proteins that might otherwise interfere with clear results (SN: 7/26/86, p.56). Moreover, because extremely specific peptides can be produced, there is at least a theoretical possibility of differentiating between closely related viruses, such as HIV-1 and HIV-2, that have slightly different protein structures. HIV-1 is the cause of AIDS in North America, while HIV-2 is more common than HIV-1 in some parts of Africa and is becoming increasingly prevalent in some European countries. Researchers know little about the clinical significance of the viruses' differences. Current ELISA tests for HIV-1 are estimated to be only 30 to 85 percent effective in spotting antibodies to HIV-2, and no HIV-2 test is approved for use in the United States.

Other advantages of synthetic or recombinant HIV peptides include greater control over lot-to-lot variation of the test product, test results in less than five minutes and the possibility of creating a product that could test simultaneously for several different antibodies, each of which might occur at different stages of infection. Another major advantage of second-generation AIDS tests is that no live cultures of the AIDS virus are required for their production. This eliminates the chances of infection among laboratory workers where the tests are manufactured.



SmithKline Beckman

A test plate is positioned on the sampling station of a new system, introduced last month, that uses genetically engineered antigens to detect AIDS antibodies.

Scientists seeking to develop these new AIDS tests initially faced one overriding question: Which of the many peptides making up the AIDS virus are most responsible for stimulating an antibody response in the body, and so would prove most useful as an antibody "probe?" Their decision was important, as the AIDS virus shows great diversity and only a peptide found in essentially *all* AIDS strains could ensure a minimal number of false negative results.

Had the AIDS epidemic occurred even 10 years before it did, this task would have been impossible. But recent advances in genetic engineering have made scientists remarkably adept at mapping the amino acid sequences of various peptide chains, comparing them to each other and mass-producing the peptide fragments that are of most interest.

In the case of the AIDS virus, researchers quickly turned their attention to two or three regions of the viral genome, or genetic blueprint. These included two regions coding for peptides that make up the viral jacket, or envelope, and one region important for the development of the so-called core protein. These peptides were found to be highly immunogenic, or capable of stimulating antibody responses. Moreover, their amino acid sequences proved to be remarkably constant among the many different strains of HIV-1 — an important quality if the test is to detect all varieties of HIV-1, and evidence that the peptides are critical to viral survival.

Researchers at a number of pharmaceutical companies and research institutes set about mass-producing these peptides, sometimes varying their structures in minor ways to identify the parts most critical to antibody binding. Some companies produced the peptides one amino acid at a time in chemical solutions, while others engineered bacteria or yeast to produce the peptide pieces. They then tested the synthetic or engineered peptides to see if they were immunologically equivalent to their naturally occurring counterparts. Researchers have yet to show this equivalence, as naturally produced peptides have chemical side chains added to them in the production process and may have three-dimensional structures somewhat different from those of laboratory-derived peptides.

Because so little is known about the immunochemistry of synthetic peptides, and of AIDS peptides in particular, the FDA has been cautious in

Home AIDS tests

Several companies have reported that they are developing either mail-in or do-it-yourself AIDS test kits, but FDA approval is not likely to come soon. The FDA's blood products advisory committee has announced that such tests will require a formal premarket approval process — generally a long and expensive affair. Even then, there may be insurmountable problems in getting the tests approved.

Among the FDA's concerns about home kits: the lack of face-to-face counseling following test results; the need to collect enough blood to allow confirmatory testing; the need to ensure the safe disposal of lancets used to prick one's own finger; and the need to ensure proper physician follow-up. For mail-in tests, which would involve collecting blood at home and sending in the blood sample for anonymous testing, there are additional concerns. Foremost among them is the need to design mailers that prevent blood samples from contaminating postal workers.

— R. Weiss

its review of second-generation AIDS-test data. According to Kenneth P. Seamon, acting chief of molecular pharmacology at the FDA's Center for Biologics Evaluation and Research (CBER), a number of issues remain unresolved even as the FDA begins to consider license applications for these new products. It is still not clear, for example, how pure these peptides should be, whether three-dimensional analysis should be required on a lot-to-lot basis and whether it matters if certain side chains have minor chemical variations. Such variables may affect the rate of false positive or false negative results, he says.

The problem of test validation is especially tricky because the new tests may be more sensitive than any of the current standards. Thus, if a Western blot comes up negative and the new test comes up positive, it can be difficult to know whether the former is a false negative or the latter a false positive. Indeed, it may take months for a patient to definitively convert to a positive antibody status, finally implying that the new test was in fact detecting early evidence of infection. (Antigen tests that screen for the presence of the AIDS virus itself, and that might confirm antibody results one way or the other, are still experimental.)

It's likely, say FDA officials, that licensing requirements will vary depending on the populations to be targeted by the new tests. AIDS testing is no longer limited to blood donor screening, but is increasingly used by physicians, insurance companies and employers. "A few false positives are not a problem in a large-scale screening program, so long as the test flags any possible positives," says Jay S. Epstein, chief of the retrovirology laboratory at CBER. "Conversely, making the wrong diagnosis in the clinical setting can be a disaster."

In addition to the need for highly specific tests, Epstein says, the FDA will balance other special needs for particular applications. For example, there is an increasing need for rapid tests for emergency room physicians and surgeons who want quick analyses of their patients' possible infectivity. Simplicity of testing is also becoming more important as a variety of companies begin to apply for approval of mail-order or "do-it-yourself" AIDS tests (see box). These applications of new test technology, says Epstein, "raise a lot of special issues in terms of divorcing the actual testing from the medical setting and proper counseling."

DIAGNOSTICS IN DEVELOPMENT

Test Name	Manufacturer	Indication	Development Status
to be announced	Abbott Labs	detects HIV antigens	pending FDA approval
Envacor	Abbott Labs	detects antibodies to core antigen p24 and the envelope antigen p41	pending FDA approval
Recombigen Latex HIV (rapid HIV antibody test)	Cambridge Bioscience	detects HIV antibodies	pending FDA approval
Recombigen EIA HIV (two-hour immunoassay)	Cambridge Bioscience	detects HIV antibodies	pending FDA approval
SureCell	Cetus, Eastman Kodak	detects HIV antibodies	in development
to be announced	Cetus, Eastman Kodak	amplifies and detects HIV viral DNA	in development
RIBA HIV216	Chiron	validates results of positive ELISA test	clinical trials
HIV p24 core antigen test	DuPont	detects HIV p24 core antigen	pending FDA approval
rapid HIV antibody test	DuPont	detects HIV antibodies	clinical trials
VIRGO HIV IFA (immunofluorescence assay)	Electro-Nucleonics	detects HIV antibodies	pending FDA approval
to be announced	Gen-Probe	test for AIDS virus	early research stages
to be announced	Hoffmann-La Roche	detects HIV antibodies	pending FDA approval
MGSearch HIV-160	MicroGeneSys	detects HIV antibodies	clinical trials
to be announced	Syntex/Syva, Cambridge Bioscience	test for AIDS antibodies	in development
to be announced	Syntex/Syva	test for AIDS virus	in research
Fluorognost (immunofluorescence assay)	Thermascan	HIV-1 antibody confirmation test	approved as Investigational New Drug; clinical trials
to be announced	Viral Technologies (Interleukin-2, Alpha-1 Biomedicals)	detects HIV p17 antibodies	in development

Adapted from Pharmaceutical Manufacturers Assoc.

Test simplicity especially appeals to developing nations, which often lack the equipment and trained technicians to perform the more sophisticated ELISA and Western blot tests. According to Thomas C. Quinn, of the Johns Hopkins University School of Medicine in Baltimore, it is not uncommon to find 6 to 18 percent of blood donors infected with the AIDS virus in some African countries — "an astoundingly high rate compared to that in the United States," where the rate is a small fraction of a percent. Blood transfusions are common in Africa for the treatment of malarial anemia, Quinn notes, and the lack of screening tests is resulting in thousands of new AIDS cases every year, many of them in children.

With the availability of experimental, second-generation AIDS tests, some of which require no electricity to perform and are stable for months in tropical climates, that contamination rate promises to drop. However, Quinn and others note, such tests — which are interpreted by technicians rather than scored by machine — are only as accurate as the technicians who read them.

Indeed, says Girish N. Vyas, of the University of California School of Medicine in San Francisco, the new tests are so accurate that the greatest risk of error even among highly trained technicians is from mislabeled specimens and incorrectly transcribed results. "We have reached a point in technology where the human error rate is higher than the technical error rate," he says.

In an attempt to minimize technician error, Vyas and his colleagues are developing a "flow cytometry" blood analyzer that contains immunoreactive beads coated with recombinant AIDS antigens in a completely closed system. The system will identify samples by bar codes, withdraw blood from specimen tubes, test for AIDS antibodies and interpret and print out results without human intervention at any of the steps. Still, he notes, there is room for error in the initial collection and labeling of blood samples.

Not everyone is convinced that the new AIDS tests will prove significantly more accurate than current tests. "Our study is still finding a few false negatives on the recombinant antigen and synthetic peptide tests," says Michael S. Ascher, deputy chief of the California Department of Health's Viral and Rickettsial Disease Laboratory. "Whether these are significant or not remains to be seen." Ascher's laboratory has been conducting independent analyses of some of the new tests, and he says current tests, if done repeatedly, may be as accurate as the new tests.

However, most others who attended the recent workshop in Bethesda seemed convinced that the new tests have many advantages over current tests. "The level of performance that we have seen is at least as good as and perhaps better than the Western blot," says Donald Burke, chief of the Department of Virus Diseases at Walter Reed Army Institute of Research in Washington, D.C. Burke has overseen the screening of military applicants and active-duty personnel conducted by the Department of Defense since 1985. More than 2 million tests have been performed in that program, which has been using an experimental second-generation test as a confirmatory test in conjunction with the Western blot. Burke estimates that by using the new tests the Army has lowered its false positive rate from approximately 1 in 100,000 to less than 1 in 250,000. The number of inconclusive results has also dropped significantly, he says.

Several researchers also report that the newer tests are detecting so-called seroconverters—individuals who are just beginning to show evidence of antibodies—weeks or months earlier than current tests. "Seroconverters are the most difficult to detect and are the greatest challenge to any screening assay," says Gerard W. Robey, of Abbott Laboratories in Abbott Park, Ill.

Although more research needs to be done, detection of early disease may require manufacturers to include antigens able to detect specific "early disease" antibodies that in some cases disappear with the later stages of disease. There is still some debate about which antigens will prove most effective, however, and the problem is exacerbated by the relatively high mutation rate the AIDS virus is showing. Indeed, scientists comparing the amino acid sequences of various strains of HIV over the past several years have noted with some alarm the increasing variability of HIV's molecular construction.

"We're inclined to conclude...that the human immunoviruses have made a recent ecological and evolutionary breakthrough, that they are mutating at a very brisk pace and that we must expect the diversity of these viruses with respect to gradations of their pathogenicity to continue to expand for some time," says Gerald Meyers, director of the Human Retroviruses and AIDS Database Project at Los Alamos (N.M.) National Laboratory.

Meyers and others warn that highly specific, second-generation tests will have to contain predictably immutable peptides—ones the virus absolutely must have to survive. However, he says, recent research is suggesting that mutation locations will be very hard to predict. He says current mathematical models show that perhaps 30 to 40 percent of the viral-envelope genetic code can mutate without affecting viral survival. But the specifics of *which* 30 to 40 percent may vary with each subtype. And depending upon which mutations actually occur, the rules are reshuffled as to which further mutations may occur next.

"As mutations become fixed there will be concomitant changes in the positions that are available for changes," Meyers says. "Ironically," he adds, bringing his mathematical model back to biomedical reality, "I understand that this is what mathematicians call contagiousness." □