A Rebel Without a Cause of AIDS

Biologist Peter Duesberg has gained a lot of public attention and stirred up the wrath of many former colleagues with his claims that HIV is not the culprit behind AIDS

PETER Duesberg does not believe that AIDS is caused by any microbe known to man, especially not the human immunodeficiency virus called HIV. Says Duesberg: "That virus is a pussycat." So sure is Duesberg that AIDS is not caused by HIV that the professor of molecular biology from the University of California at Berkeley is telling reporters that he would gladly be injected with the virus—as long as the concoction is not prepared at the bench of Robert Gallo, the researcher from the National Cancer Institute who is the co-discoverer of HIV.

Basically, Duesberg does not think that HIV is virulent enough to cause AIDS, a conclusion he bases on widely recognized gaps in knowledge about how the virus operates in the body. His unsettling offer to inject himself with HIV and his pointed jabs at fellow scientists have aroused a great deal of anger and exasperation among AIDS researchers, who insist that an overwhelming body of evidence points toward HIV as the culprit behind AIDS. At the same time, Duesberg's remarks have won for the professor a large amount of media attention, particularly in the gay press where he is something of a hero and where government types such as Gallo are often portrayed as villains or fools.

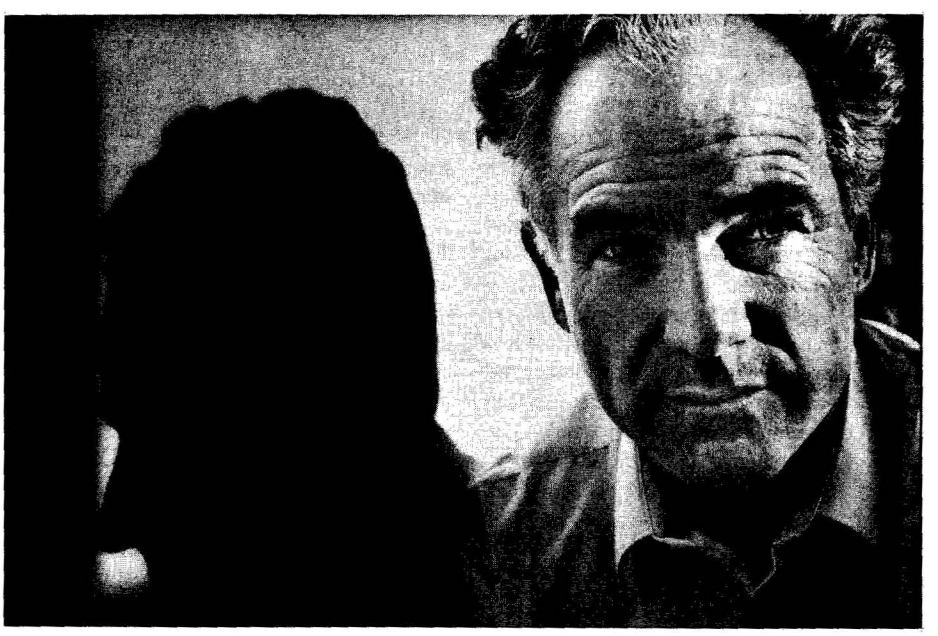
For his part, Duesberg is well suited to the role of iconoclast, and indeed, he has played the part of the gadfly before. Immensely quotable, with a sharp sense of humor and a slight Germanic accent, the 51-year-old professor does not hesitate to tweak the noses of figures in the biomedical research community whose egos often loom larger than life. Yet Duesberg is not an iconoclast without credentials. He is a legitimate investigator. A member of the National Academy of Sciences, Duesberg did pioneering work in the field of viruses and cancer-causing genes in the 1970s. Moreover, Duesberg is an insider, a colleague and sometime friend of the same researchers he now attacks.

None of this, however, has gotten Duesberg a formal response to the article he wrote for the peer-reviewed journal *Cancer Research* in March 1987, in which Duesberg first stated his objections to the HIV orthodoxy. "Why won't they respond to me?" Duesberg constantly asks reporters, who then ask AIDS researchers the same question.

"I cannot respond without shrieking," says Gallo when confronted with one of Duesberg's statements. "It is absolute and total nonsense," says Anthony Fauci, coordinator of AIDS research at the National Institutes of Health (NIH). "Irresponsible and pernicious," says David Baltimore, director of the Whitehead Institute in Cambridge, Massachusetts, and a chairman of the Institute of Medicine–National Academy of Sciences committee that produced the bench mark report *Confronting AIDS*.

Yet Duesberg keeps pressing. "Like a little dog that won't let go," says Gallo. And so a debate of sorts has been lurching along, staged in the most unlikely forums. In January, for example, *Spin*, a rock music magazine produced by the son of *Penthouse* publisher Bob Guccione, ran a question-and-answer interview with Duesberg in which he detailed his objections to HIV and accused "the AIDS establishment" of collusion and intellectual bankruptcy, suggesting that because two leading AIDS investigators have a financial interest in a company that produces diagnostic kits to test for antibodies to HIV, they were incapable of questioning their own AIDS research. (In the world of biomedical research, where ties to industry are pervasive but mentioning the fact is not, these are fighting words.) The following month, *Spin* published a bizarre interview with Gallo, in which the scientist spent half of the piece ranting and raving about the stupidity of Duesberg's statements, while punctuating his remarks with the occasional expletive. (The interview was tape-recorded without Gallo's knowledge.)

In the midst of all this, Jim Warner, a policy adviser in the White House, suddenly became interested in retrovirology. Warner wanted the White House to co-host a meeting organized by Harvey Bialy, an editor at the journal *Bio/Technology*, at which Duesberg would take on someone from NIH. Scheduled for January, the meeting was canceled. Columnists Jack Anderson and Joseph Spear then wrote an article in February in which they chided Gallo for refusing to defend his ideas. The column stated that Duesberg reached his "fresh point of view" after studying HIV in Gallo's laboratory, insinuating that some kind of conspiracy of silence was afoot. Gallo correctly points out that Duesberg has never studied HIV and has never worked in his lab. In fact, Duesberg has not done a single experiment in the AIDS field. As for the proposed White House affair, Gallo claims he only heard



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Peter Duesberg *maintains that AIDS is not caused by an infectious agent but by sexual excess and drug abuse.*

lope for the virus. These envelope molecules may erupt from the infected cell's membrane and bind to special receptor sites on other cells, causing the formation of giant constellations of cells called syncytia. In this way, one infected cell could disable as many as 100 uninfected cells.

There is also some indication that an autoimmune response might be at work, whereby the body's immune system targets for destruction some of its own cells that have been subtly altered by the virus. For example, soluble gp120 might be secreted by infected cells and become attached to uninfected cells, thereby setting them up as targets for attack by "killer" T lymphocytes. The virus may also be infecting populations of precursor cells which give birth to T cells, says Gallo. Or viral products may be directly toxic.

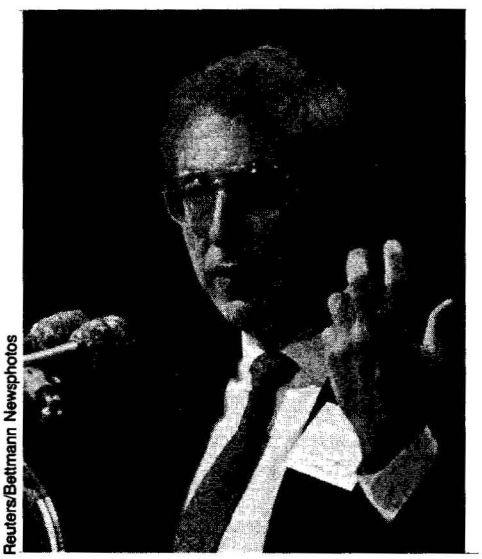
In addition, Gallo and others point out that T cells are not the only targets of HIV: monocytes and macrophages are also infected. Macrophages, which appear to sequester the virus, may prove extremely important in disease progression. Researchers also report that HIV may be passed from cell to cell. Free virus particles may not even be necessary. Again, no one is certain. No one knows exactly how HIV causes the gradual depletion of T cells seen in AIDS. It is a mystery of the most intense interest. But the questions that Duesberg raises about HIV are not novel ones, say AIDS researchers.

"We don't know how the virus is transmitted. Is it free virus particles or do you have to exchange cells? We don't know the initial targets. Are they lymphocytes or macrophages? We don't know where the virus is in the body during the initial stage of infection or during the long period when a person is antibody-positive but still asymptomatic. These are all important issues, but just because we don't know all the answers doesn't mean that we can't extrapolate from pretty good data that keeps pointing toward HIV," says Malcolm Martin, chief of the laboratory of molecular biology at the National Institute of Allergy and Infectious Disease.

AIDS investigators say that it is important to remember that the depletion of T cells is gradual. According to Fauci, when a person is first infected with the virus, there is a big burst of viral replication that precedes the production of antibodies against the invading microbe. During this early, acute stage, a person with HIV often gets symptoms similar to the flu. With the onset of antibodies, the virus appears to be inhibited. But the virus persists. Over time, as anti-HIV immunity wanes, the virus continues to replicate in bursts, perhaps activated by as yet unknown signals, which may be other viral infections or the workings of the immune system itself.

Says Gallo: "Everything Peter says about pathogenesis [how a pathogen causes disease] is irrelevant because no one in history has ever had to explain pathogenesis to explain cause. We don't know the detailed pathogenesis of cholera, or tuberculosis, or when you get hit by a truck. It's wonderful if you can explain every molecular change all the way down the line and make it all make sense. But you don't have to. That said, let me add that we understand about as much of the pathogenesis of this disease as we know for most diseases."

As for Duesberg's statement that the virus can be isolated in only 15% of people with antibodies to HIV, the figure comes from an early paper published by Gallo and colleagues (*Science*, 7 December 1984, p. 1165), in which they examined fresh tissue from 65 patients with AIDS or ARC. They found integrated genetic material from HIV in only 9 of the 65 tissue samples. Gallo states that the point of the paper was not to try to isolate virus from all 65 patients, but to see in which tissue samples they could find cells infected with HIV.



Reuters/Bettmann Newsphotos

Robert Gallo *cannot respond to Duesberg's statements "without shrieking."*

Duesberg, however, uses these findings to state that HIV is not present in all stages of the illness, meaning that the virus violates the first postulate of Robert Koch, the eminent German bacteriologist who in the 1880s discovered the bacilli that cause tuberculosis and cholera. Koch's first postulate stipulates that for an organism to be considered the cause of a disease it must be present in all stages of disease.

Today, Gallo maintains that a good laboratory can isolate the virus from between 80 and 100% of all persons with antibodies to HIV, including patients with AIDS or ARC. "We're damn close to 100%," says Gallo. With the introduction of gene amplification techniques now being developed, most AIDS researchers say that the difficulty of isolating virus from persons with HIV will be overcome. To this, Duesberg responds: "It doesn't matter if the techniques become more and more sophisticated. There still isn't enough virus to be clinically relevant."

Duesberg keeps pushing. Not only is HIV too inactive to cause AIDS, he maintains that HIV acts like "no known virus" because of its long latency and the fact that it persists despite the production of antibodies.

For most people, learning that their blood contains antibodies to the "AIDS virus" is a traumatic and frightening event. But Duesberg says that such persons should be "congratulated." "Hurrah, your body has won!" says Duesberg. "A cardinal rule in virus infection is that viruses cause disease . . . before immunity and not after immunity. The host, in other words, has to be permissive to the virus to let it happen. If you are not permissive, that is to say, if you have antibodies, the virus doesn't have a good chance to cause disease."

Martin retorts: "This is ignorance." Martin says there are many kinds of antibodies and that the mere presence of antibodies does not equal protection against HIV or any other pathogen. "We know in the lab that some antibodies will bind to the virus particles, some antibodies will immunoprecipitate, some antibodies will neutralize particles, others won't," says Martin.

As for HIV's long latency, Duesberg contends that all known viruses cause disease soon after infection. Says Duesberg: "Shortly after exposure to a virus, you develop symptoms or you don't. If you're lucky, you don't. If you're unlucky, you do. . . . That is to say that viruses work quickly or not at all. Again, the AIDS virus seems to be the exception to the rule."

Yet there are other viruses besides HIV that have long periods between infection and disease, says Bernard Fields, chairman of the Department of Microbiology and Molecular Genetics at Harvard Medical School and editor of the textbook *Virology*. The herpes family, for example, "is notorious for its long latency, despite the presence of antiviral antibodies," says Fields. Herpes simplex virus, for one, continues to causes cold sores and blisters years after infection. The varicella-zoster virus that is responsible for chickenpox also takes up residence in the sensory ganglia and years later produces shingles. The measles virus is responsible not only for the acute disease with its red

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To: Jim Liverman / Jack Kanarski

From: John Fraser

Re: AIDs Diagnostic Tests

C + E News article attached as requested.

NOVEMBER 23, 1987

CHEMICAL & ENGINEERING NEWS

A C&EN SPECIAL ISSUE

AIDS The science of a human tragedy

John Frazer

The Problem of Diagnostic Tests

Deficiences of current HIV tests are being addressed by development of new kinds of tests for antibodies, antigens, and nucleic acids

Ron Dagani, C&EN Washington

In a perfect world, a person infected with human immunodeficiency virus (HIV) would always show up positive in a blood test; an uninfected person would always show up negative. What could be simpler?

Unfortunately, life is not simple. And present-day tests to detect HIV infection are far from perfect. Some individuals who aren't infected show up positive. Such a result, called a false positive, can lead to needless anxiety and depression, problems with interpersonal relations, discrimination, loss of home and job, or even suicide. That's because persons who harbor the virus are likely to develop—and die of—AIDS. On the other side of the fence are those infected individuals who falsely test negative. They breathe a sigh of relief and may proceed, unknowingly, to infect others, either by donating blood, engaging in unprotected sex, or sharing a needle to inject street drugs. Simply put, false positive and false negative test results destroy lives.

With wider HIV testing looming in the future, medical researchers are striving to eliminate such misleading test results by making HIV diagnostic tests more sensitive and more specific. Sensitivity is the ability to detect low levels of a target molecule; specificity is the ability to detect the target molecule exclusively. In addition, researchers are developing new tests that will be quicker, simpler to perform, and less expensive than those currently available.

The tests now in use or under development are of two basic types. The type that came into use first is an indirect test: It detects antibodies (proteins called immunoglobulins) that the immune system has mobilized against the invading AIDS virus. The second type of HIV test is one that detects the virus directly, either through its antigens or its genetic material.

The most widely used antibody test is the enzyme-linked immunosorbent assay (ELISA). This test can screen large numbers of blood samples. It was rushed into the marketplace to protect the blood supply, which had already been infiltrated by the AIDS virus. ELISA was purposely designed to be oversensitive—to catch even "suspicious" blood samples. As a result, it produces many false positives. In fact, according to a study by Harvard University researchers, using ELISA to screen persons at low risk of infection "yields many more false positives than true positives."

All blood samples that test positive are retested using ELISA. Those that test positive again are subjected to a different, more definitive test called the Western blot. Unlike ELISA, which is a yes-or-no test, the Western blot actually reveals the antibody profile of a blood sample. Thus, it is considered a more accurate indicator of the presence of HIV antibodies. If the Western blot is positive, the person is considered to be infected.

The Western blot produces fewer false positives than ELISA, but it's not infallible. Scientists estimate that, at the end of this testing process, one to three individuals out of 100,000 in a low-risk population will be incorrectly told that they are infected with HIV. The false positive rate is lower in groups that have a high prevalence of HIV infection, such as urban homosexual men and drug addicts.

Other assays do exist to confirm a positive ELISA, but these aren't practical for large-scale use. The radioimmunoprecipitation method, for example, is sensitive and specific, but it involves radioactivity and requires considerable technical skill. Virus culturing is the most specific method, but it is difficult, time-consuming, and also requires highly skilled personnel.

Blood banks and other HIV testing centers in the U.S. currently can choose from seven ELISA test kits that have been approved for commercial use by the Food & Drug

Technician uses Du Pont HIV antigen test to monitor viral activity

Administration. These kits are available from Abbott Laboratories, Cellular Products, Du Pont, Electro-Nucleonics, Genetic Systems, Organon Teknika, and Ortho Diagnostic Systems. Du Pont is unique in that it is the only U.S. firm whose Western blot test also has been licensed for clinical use. Du Pont's ELISA and Western blot tests were both developed in partnership with Biotech Research Laboratories of Rockville, Md. Western blot kits from other firms are being used in research and may be approved by FDA for wider use.

Assays like these detect HIV antibodies by capturing them on antigen-coated surfaces and using a color reaction to visualize the antigen-antibody complex. The commercially available ELISAs, which are called first-generation tests, use disrupted whole-virus preparations as the antigen. These preparations, though made from purified virus, are contaminated with cellular debris that can attract non-HIV antibodies and thus produce false positives.

Second-generation tests

Scientists now are developing second-generation tests that use one or more genetically engineered HIV antigens instead. Because these preparations are purer, the number of false positives is reduced. Another benefit is that the test manufacturer avoids the hazard of working with live virus.

Some of these second-generation tests are said to be so reliable and easy to use that they may allow rapid HIV screening to expand into new markets, such as developing nations, physicians' offices, and possibly even the home market. For instance, at the University of California, Davis, a team of medical researchers has developed a dot enzyme immunoassay (dot EIA) on a small plastic card. The assay detects antibody to gp41, the glycoprotein that spans the outer membrane of HIV. In this assay, a drop of serum is exposed to recombinant gp41 on the card. If anti-gp41 antibody is present, it binds to the antigen. After detection reagents are applied, the bound antibody signals its presence by the appearance of a blue dot.

According to James R. Carlson, an assistant professor of pathology and internal medicine who leads the UC Davis team, the prototype of this dot EIA was more than 99% accurate in preliminary evaluations using blood sera from foreign and U.S. sources. These results compare favorably with ELISA and Western blot results, he says. Moreover, the assay takes about 30 minutes to perform, compared with two to four hours for the typical ELISA. It's also less expensive than the ELISA—about 25 cents versus $1.00 to $4.00 for ELISA kits.

A test like the dot EIA seems to be tailor-made for remote areas that lack highly skilled technicians or laboratory facilities, such as Africa. In some African cities, as many as 18% of the blood donors have been found to be HIV-infected, Carlson says, "yet donor blood isn't usually screened due to technical and economic constraints." The test is being developed for clinical use in a joint venture between Virotechnology Laboratories of Stockton, Calif., and Bio-Medican Corp. of Huntington Beach, Calif.

An even faster second-generation test has been produced by Cambridge BioScience, a biotechnology firm based in Worcester, Mass. The basis of this test is Recombigen, a novel recombinant antigen that attracts antibodies to both gp41 and gp120, the virus's envelope glycoprotein. "All of our data show that envelope antibody is the earliest and most important marker for detecting HIV antibody," says Rod N. Raynovich, vice president for business development at Cambridge BioScience.

The firm's test involves mixing a drop of blood serum with a drop of latex reagent on a plastic card. The reagent contains microscopic latex beads coated with Recombigen. If antibodies to the envelope proteins are present in the sample, the latex beads clump. The resulting clusters become visible in minutes as small white dots. If clumping has not occurred within five minutes, the sample is considered HIV-free.

In June, Thomas C. Quinn of the Immunoregulation lab of the National Institute of Allergy & Infectious Diseases (NIAID) reported at the 3rd International Conference on AIDS in Washington, D.C., that the Recombigen latex agglutination assay had been tested on sera from 2000 patients residing in Africa and the West Indies. Those tests indicated that the assay was accurate more than 99% of the time, and was more specific than a single ELISA. Quinn also said that the latex test was faster and simpler than the ELISA, and reliable enough that confirmatory tests might not be needed. Raynovich believes the Recombigen assay is better than the Western blot, and could replace it as a backup test.

Cambridge BioScience hopes to receive government approval to begin marketing the latex test in 1988 for use in physicians' offices and other low-volume, "decentralized" testing sites. The firm began conducting clinical trials of the test in September. Raynovich says the company plans to submit the results to FDA by the end of this year. In the meantime, FDA has allowed Cambridge BioScience to ship 50,000 test kits to Zaire, where the test is being used to screen for HIV-infected blood.

Murex Corp. of Norcross, Ga., also is developing a fast HIV screening test, but this one doesn't rely on a single recombinant protein. Rather, it uses seven natural HIV antigens that have been isolated by affinity purification using monoclonal antibodies, according to Gerald A. Bush, who has been leading the development effort. These antigens react with antibodies to the three major types of structural proteins in the virus—envelope, core, and replication-related proteins.

The reaction between these purified antigens and serum antibodies takes place inside a small, disposable, plastic cartridge. A positive reaction is indicated by the appearance, within 10 minutes, of a blue color in the cartridge's window.

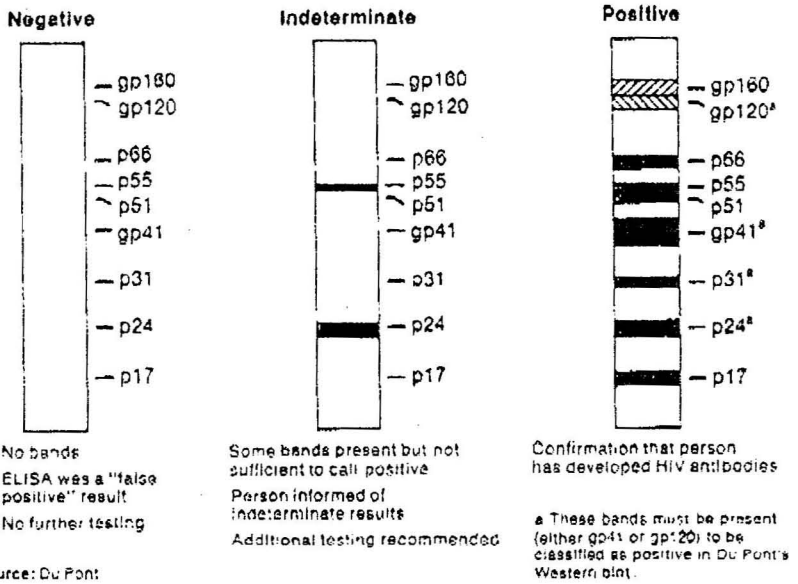
In early tests, the sensitivity and specificity of the Murex assay have been greater than 99%, says Bush. The assay actually picks up seropositive blood earlier than the ELISA, he notes. That might be because the purified antigens are so highly concentrated that they can detect lower levels of antibody, he explains. Bush says Murex expects

How ELISA and Western blot tests work

The screening of blood samples for antibodies to HIV (the AIDS virus) usually starts with an enzyme-linked immunosorbent assay, or ELISA. In this assay, blood serum is diluted and placed in microtiter wells or on beads that have been coated with HIV antigen (derived from disrupted virus that has been grown in cell culture). If the specimen contains antibodies (immunoglobulins), they will bind to the test kit's antigens. After an incubation period of about two hours, any unbound antibody is washed away. An enzyme-labeled antibody to human immunoglobulin is added, and this binds to any HIV antibodies that have become bound to antigen. The bead or well is again washed to remove any unbound antibody. A substrate is added and the enzyme bound to the antihuman antibody catalyzes a reaction that produces a color change. The optical density is measured spectrophotometrically and is directly related to the amount of HIV antibodies in the sample. The absorbance reading is interpreted by comparing it to positive and negative control samples containing known quantities of antibodies.

Samples that repeatedly test positive by ELISA are subjected to a different, more expensive procedure called the Western blot. This test identifies antibodies to the major HIV antigens. These antigens, derived from purified disrupted virus, are fractionated by size using polyacrylamide gel electrophoresis by the test's manufacturer. The fractionated viral proteins are then transferred onto nitrocellulose paper. A serum sample is applied to a strip of the paper. If antibodies to HIV are present, they will bind to the viral antigens. An enzyme-labeled antibody to human immunoglobulin is applied to the paper to bind to any HIV antibodies bound to antigen. An appropriate substrate is added and the enzyme catalyzes the color reaction. If HIV antibodies are present, a pattern of distinctive bands appears on the strip. The location of each band indicates reaction to a specific viral protein and is compared visually with a Western blot from a specimen in which antibodies to all of the HIV proteins are present. A positive result on this test almost always means the blood sample contains HIV antibodies.

Western blot readings have three possible interpretations



Source: Du Pont

to begin clinical trials of the assay by the first of the year, and the test to be licensed in 1988.

Home test for HIV?

A special feature of Carlson's dot EIA and the Cambridge BioScience and Murex rapid tests is that they work with whole blood as well as serum, according to sources involved in their development. That means they have the potential to be over-the-counter or home tests. But scientists and company officials discount this possibility, at least for now. They worry about the medical and ethical implications of AIDS testing in the home. Positive test results can devastate people, and any test could give a false positive, they say. "It's like giving somebody a death sentence," remarks one company spokesman.

Health care professionals agree that people need proper counseling to correctly interpret the results, plan confirmatory tests, and obtain medical and psychological therapy, if needed. "We believe a physician must be in the loop," says Raynovich. "We don't think FDA would approve a home test [for HIV] in the near term."

Similar thinking prevails at Du Pont, which is clinically evaluating an HIV antibody test the size of a matchbook. This test, like its potential competition, uses a color change to give the same yes-or-no information as ELISA, only much quicker (in five minutes). Du Pont spokesman Mike Ricciuto says the firm hopes to begin marketing the matchbook test in Africa later this year. In the future, he says, Du Pont might seek FDA approval to sell the test to hospitals and physicians in the U.S. "It would probably never be marketed as a home test," he adds.

Because any of these screening tests could produce false positives, most scientists believe that there's still a need for confirmatory assays such as the Western blot. This test, sometimes called the immunoblot, provides more information than the standard ELISA because it exposes a serum sample to a strip of paper containing all the key HIV antigens separated according to their molecular weight. Thus, the identity and relative abundance of specific antibodies in the sample can be

visualized on the strip according to the specific antibody-antigen reactions that occur.

Problems with Western blot

Nevertheless, indeterminate and false positive results can arise on the Western blot because the method is not standardized. A 1986 Institute of Medicine report on AIDS noted that "There has been considerable variation in what different laboratories have interpreted as positive in Western blots." In the past, some testing labs have considered antibody to the core protein p24 as sufficient for a positive result. The Institute of Medicine report states that this is an equivocal result. The National Institutes of Health requires antibody to p24 and to the transmembrane protein gp41. Du Pont says a positive result must include p24, p31 (a replication-related protein), and either gp41 or gp120.

Improvements in the Western blot have helped to some extent. Earlier versions of this assay were deficient in detecting antibodies to gp120 because this key protein was selectively lost during the manufacturing process. The newer versions of the test, such as Du Pont's, offer enhanced detection of antibodies to gp120 and its precursor protein gp160, making it easier to classify "some potentially ambiguous sera," says Stanley H. Weiss of the National Cancer Institute's environmental epidemiology branch.

Even so, the interpretation of Western blot reactions can still be problematic. Weiss notes, for example, that some persons who had "unusual Western blot reactivity patterns" or showed no evidence of HIV antibodies were found to be HIV-infected by virus culture. As a precaution, blood banks currently reject donations that repeatedly test positive by ELISA, even if they test negative in the Western blot. Many of these ELISA results certainly are false positives, and the blood that is thrown out is wasted.

In an effort to clear up the uncertainty in some Western blot readings, scientists at Abbott Labs have devised an immunoassay that is complementary to the Western blot. This assay, Envacor, actually consists of two tests: One detects antibody to an envelope protein (gp41) and the other detects antibody to a core protein (p24).

Envacor may be useful as a diagnostic tool because the two antibody types provide different information. "Envelope antibodies are present in virtually everybody who's infected," says Jean-Pierre Allain, who manages Abbott's research on AIDS diagnostics. They are "an extremely stable and reliable marker for HIV infection" throughout the course of the disease, he adds. Antibodies against p24, by contrast, are not always present and their level changes depending on the stage of infection. About 85 to 90% of infected asymptomatic persons have anti-p24 antibodies. But as these individuals become sicker, anti-p24 levels drop lower and lower, eventually becoming undetectable. Thus, Envacor might be useful in charting and predicting the immunological course of the disease, says Carlson, who directs the AIDS Virus Diagnostic Laboratory at UC Davis.

The Abbott test offers a number of advantages over the Western blot, according to Allain. Envacor is easier to perform because it's in an ELISA format. It gives a numeric readout of results, thus avoiding the subjective interpretations required by Western blot. It's also quicker, although it requires an overnight incubation step like the Western blot.

Allain: immune system collapse

University of California's Carlson holds the dot EIA test for HIV

On average, the two tests are of similar sensitivity. But Envacor's specificity is very high—99.9%. This means that, statistically, 999 out of 1000 HIV-negative blood samples will be unreactive in the test. Only one in 1000 is likely to turn up as a false positive. In Europe, where Envacor has been in use for more than a year, Abbott recommends that it be used in conjunction with the Western blot.

Tests for viral antigen

The variability of the immune system's antibody response to HIV proteins in different people has led researchers to develop a third generation of AIDS tests, which detect the virus or its fragments directly. This approach promises to catch some of the false negative results that crop up in antibody tests.

False negatives occur in persons who haven't yet developed detectable levels of HIV antibodies. Typically, antibodies aren't detectable in blood until six to 10 weeks after infection, according to Jaap Goudsmit, head of the retrovirus laboratory at the University of Amsterdam. A recent study of homosexual men in Finland has found that some HIV-infected persons don't develop antibodies for a year or more. And some HIV carriers may never mount an antibody response at all. Even so, these individuals are still considered infectious, and their blood may contain detectable levels of antigens.

Obviously, one way to pick up infected blood earlier than is possible with antibody tests would be to look for viral antigens. Several companies are developing such antigen tests for marketing. Abbott and Du Pont unveiled their entries in June at the 3rd International Conference on AIDS. Although their antigen tests aren't yet available commercially in the U.S., they have been used as research tools for at least a year.

The Abbott and Du Pont antigen tests are both enzyme immunoassays that directly detect the core protein p24, which is shed by the virus. The Abbott test, for instance, uses purified, naturally occurring antibodies from an infected individual. These are bound to a solid phase and are allowed to capture whatever antigen may be in the sample. The visualization chemistry is similar to that used in other enzyme immunoassays.

Researchers stress that such direct-virus tests will complement, not replace, antibody tests. That's because the levels of antigens and antibodies change with time, and looking at only one or the other can give a misleading result. For example, if a person is producing antibody, then antigen in the blood will be masked to some extent by the binding of the two, Du Pont's Ricciuto explains. In this instance, antigen may not be detected at all, although an antibody assay would correctly signal HIV infection. Hence, it would be necessary to screen blood for both antibody and antigen, he says.

The rationale for adding the antigen test to blood screening programs is to eliminate donations of infected but antibody-negative blood. However, no one really knows whether this test will be able to pick up infected blood early enough to warrant the additional cost, says Ricciuto. According to John P. Phair, professor of medicine at Northwestern University Medical School in Chicago, since blood screening for HIV was instituted in 1985, scientists have documented one case in which HIV was transmitted by transfusing antibody-negative blood. Phair noted in a recent editorial in the *Journal of the American Medical Association* that blood banks would have to spend at least $5.00 to $7.00 per unit of blood to test for antigen. The added expense is not justified at this time, he concluded.

The antigen test might be more important as a way to diagnose AIDS earlier than is now possible. And

Diagnostic tests being developed for other retroviruses

In the U.S., as far as anyone knows, all cases of AIDS have been caused by the same retrovirus—a human immunodeficiency virus (HIV-1).

In Africa, the situation is more complicated. HIV-1 is widespread in Zaire and other central African nations. But a closely related human retrovirus, HIV-2, is endemic in Senegal and several other countries in Western Africa. HIV-2 appears to cause an AIDS-like disease. Both HIV-1 and HIV-2 are highly prevalent in at least two West African countries. Furthermore, Africa also is the home of simian immunodeficiency virus (SIV), which appears to be more closely related to HIV-2 than to HIV-1. SIV doesn't infect humans, but it does cause an AIDS-like disease in some monkeys.

The prevalence of infections caused by these viruses in Africa appears "catastrophic," according to a research team that includes Erling Norrby of the Karolinska Institute in Stockholm and Richard Lerner of the Research Institute of Scripps Clinic in La Jolla, Calif. They say there is "an emergency need" for simple, inexpensive, sensitive, and specific tests that can identify antibodies to these retroviruses, and distinguish between them.

Several groups have been working to develop such immunoassays, and at least two—Norrby's and a different group headed by John W. Gnann Jr. of Scripps Clinic—recently reported success. Both groups have based their assays on synthetic peptide antigens derived from the transmembrane glycoproteins of the different viruses [*Nature*, **329**, 248 (1987); *Science*, **237**, 1346 (1987)]. These antigens were incorporated into the standard laboratory screening format known as ELISA. Besides pointing the way toward better diagnosis of AIDS infections, early results from this research offer hope that it will be possible to solve what Gnann's team calls "the epidemiologic puzzle of AIDS in Africa."

Researchers also have been testing blood from American donors to check if HIV-2 has spread to the U.S. So far, no evidence of this has turned up. For now, it seems, HIV-2 tests will remain for investigational use only.

Scientists and health officials are more concerned that another insidious retrovirus may be spreading through the U.S. blood supply—HTLV-1, or human T-cell lymphotropic virus, type 1. This virus causes adult T-cell leukemia in about 1% of those infected. Like AIDS, this cancer is fatal and may not appear until years after infection. HTLV-1 is transmitted in the same way as HIV—through sexual contact, exchange of blood by hypodermic syringes, and transfusion of infected blood or blood products. Evidence indicates that many drug addicts in the U.S. have already been infected with HTLV-1, which is also implicated in other diseases.

Cellular Products, a Buffalo, N.Y., biotechnology company, and Du Pont, in collaboration with Biotech Research Laboratories of Rockville, Md., have produced experimental test kits to detect HTLV-1. The American Red Cross has been using these tests to look for evidence of the virus in blood donated by people around the U.S. The results of this study aren't yet available.

Nevertheless, many companies already see the writing on the wall. At present, all blood donations in the U.S. are screened for HIV antibodies and "we believe it is only a matter of time" before blood screening for HTLV-1 also will be required, says John R. Zeman, vice president and general manager of Eastman Kodak's clinical products division. Kodak plans to market a test manufactured by Cellular Products that can simultaneously detect HIV and HTLV-1 antibodies. Du Pont, too, is already gearing up to produce millions of HTLV-1 antibody test kits a year.

because p24 and anti-p24 levels in blood change as the disease progresses, the antigen test, like Abbott's Envacor assay, may be useful in predicting the course of the disease. Goudsmit says detectable p24 can appear as early as two weeks after infection. Weeks later—estimates range from six to 12 weeks after infection—core antigen disappears, perhaps overwhelmed by a rising tide of antibodies against it. But in some persons who go on to develop full-blown AIDS, researchers say, p24 reappears later, signaling the renewed activity of the virus. Because antigen complexes with antibody, a rising level of p24, coupled with a falling level of anti-p24, "reflects the failure of the immune defenses to contain the virus," Allain says.

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Certainly, the antigen test is expected to be an essential tool for monitoring the efficacy of experimental anti-HIV drugs by measuring antigen levels after drug administration. According to Paul A. Volberding, who directs AIDS activities at San Francisco General Hospital, the Abbott antigen test helped researchers determine that the drug zidovudine (popularly known as AZT) retards the virus's replication in the body. This was seen in declining levels of p24, which is used as a marker of viral burden.

In some HIV-infected persons, the virus retreats into the cells and lies dormant for months or years until it is reactivated. During this latency period, the virus cannot be cultured from the blood, and neither viral antigens nor antibodies are detectable. The only way to unmask the virus is to find the telltale genetic sequence it has surreptitiously inserted into the DNA of host cells.

Researchers are applying nucleic acid probes to accomplish this mission. Such a probe consists of a synthetic sequence of nucleotides—either RNA or DNA—that is complementary to the target viral sequence. When the probe encounters its target, the two strands hybridize, or join together to form a double-stranded segment. Because the probe sequence is tagged with a radioactive or nonradioactive label, the site of the target sequence can be located.

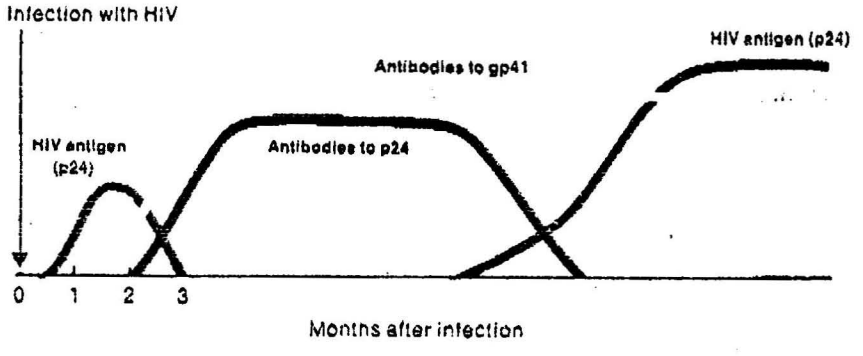
Nucleic acid probes theoretically could detect HIV infection even earlier than antigen tests. Practicably, though, many problems exist. For example, it's estimated that only one in 10,000 cells typically is infected and produces viral RNA protein. Moreover, infected cells carry only a limited number of copies of the viral sequence (usually one to three). At very early stages of infection especially, a probe would have to be extremely sensitive to pick up such small amounts of viral DNA.

Researchers at Cetus Corp., a biotechnology firm in Emeryville, Calif., have gotten around this problem by developing a method to amplify the viral DNA sequence a million times (C&EN, April 21, 1986, page 8). John J. Sninsky, director of diagnostics at Cetus, explained it this way to a newspaper reporter: "If looking for the AIDS virus is [like] looking for a needle in a haystack, our procedure allows you to make a million needles." Cetus claims the procedure can detect viral genes if present in only one of every 5000 cells.

Even so, the use of such probes requires a more sophisticated lab setup than do most other HIV tests. This is limiting the use of these probes. Furthermore, some scientists believe that the promise of this tool will not arrive until "somebody figures out a way to automate it," says Du Pont's Ricciuto.

Although researchers now can test for antibodies, antigens, and even bits of viral genes, they still don't have a clear idea of what the test results mean in terms of the patient's clinical condition and prognosis. Perhaps, one diagnostics analyst tells C&EN, that all-important connection will be made once scientists have in place a battery of assays that can be run routinely on AIDS patients. ◻

Core antigen, antibody levels vary as disease progresses



A hypothetical patient's profile shows the inverse relationship between two key HIV markers—the core antigen p24 and antibodies raised against it. p24, which appears in blood soon after infection, is neutralized by a rising tide of anti-p24 antibodies. As long as the level of these antibodies remains high, the patient's condition is stable. But months or years later, when the virus starts replicating again, its stepped-up production of p24 overwhelms the immune defenses by complexing all the available anti-p24 antibodies. By contrast, the level of antibody to the transmembrane glycoprotein gp41 remains fairly constant

Source: Abbott Laboratories

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(919) 493-0101

To: Jim Liverman / Jack Kamarchi

From: John Fraser

Re: AIDs Diagnostic Tests

C + E News article attached as requested.

NOVEMBER 23, 1987

CHEMICAL & ENGINEERING

NEWS

A C&EN SPECIAL ISSUE

AIDS The science of a human tragedy

John Fraze

The Problem of Diagnostic Tests

Deficiences of current HIV tests are being addressed by development of new kinds of tests for antibodies, antigens, and nucleic acids

Ron Dagani, C&EN Washington

In a perfect world, a person infected with human immunodeficiency virus (HIV) would always show up positive in a blood test; an uninfected person would always show up negative. What could be simpler?

Unfortunately, life is not simple. And present-day tests to detect HIV infection are far from perfect. Some individuals who aren't infected show up positive. Such a result, called a false positive, can lead to needless anxiety and depression, problems with interpersonal relations, discrimination, loss of home and job, or even suicide. That's because persons who harbor the virus are likely to develop—and die of—AIDS. On the other side of the fence are those infected individuals who falsely test negative. They breathe a sigh of relief and may proceed, unknowingly, to infect others, either by donating blood, engaging in unprotected sex, or sharing a needle to inject street drugs. Simply put, false positive and false negative test results destroy lives.

With wider HIV testing looming in the future, medical researchers are striving to eliminate such misleading test results by making HIV diagnostic tests more sensitive and more specific. Sensitivity is the ability to detect low levels of a target molecule; specificity is the ability to detect the target molecule exclusively. In addition, researchers are developing new tests that will be quicker, simpler to perform, and less expensive than those currently available.

The tests now in use or under development are of two basic types. The type that came into use first is an indirect test: It detects antibodies (proteins called immunoglobulins) that the immune system has mobilized against the invading AIDS virus. The second type of HIV test is one that detects the virus directly, either through its antigens or its genetic material.

The most widely used antibody test is the enzyme-linked immunosorbent assay (ELISA). This test can screen large numbers of blood samples. It was rushed into the marketplace to protect the blood supply, which had already been infiltrated by the AIDS virus. ELISA was purposely designed to be oversensitive—to catch even "suspicious" blood samples. As a result, it produces many false positives. In fact, according to a study by Harvard University researchers, using ELISA to screen persons at low risk of infection "yields many more false positives than true positives."

All blood samples that test positive are retested using ELISA. Those that test positive again are subjected to a different, more definitive test called the Western blot. Unlike ELISA, which is a yes-or-no test, the Western blot actually reveals the antibody profile of a blood sample. Thus, it is considered a more accurate indicator of the presence of HIV antibodies. If the Western blot is positive, the person is considered to be infected.

The Western blot produces fewer false positives than ELISA, but it's not infallible. Scientists estimate that, at the end of this testing process, one to three individuals out of 100,000 in a low-risk population will be incorrectly told that they are infected with HIV. The false positive rate is lower in groups that have a high prevalence of HIV infection, such as urban homosexual men and drug addicts.

Other assays do exist to confirm a positive ELISA, but these aren't practical for large-scale use. The radioimmunoprecipitation method, for example, is sensitive and specific, but it involves radioactivity and requires considerable technical skill. Virus culturing is the most specific method, but it is difficult, time-consuming, and also requires highly skilled personnel.

Blood banks and other HIV testing centers in the U.S. currently can choose from seven ELISA test kits that have been approved for commercial use by the Food & Drug

Technician uses Du Pont HIV antigen test to monitor viral activity

Administration. These kits are available from Abbott Laboratories, Cellular Products, Du Pont, Electro-Nucleonics, Genetic Systems, Organon Teknika, and Ortho Diagnostic Systems. Du Pont is unique in that it is the only U.S. firm whose Western blot test also has been licensed for clinical use. Du Pont's ELISA and Western blot tests were both developed in partnership with Biotech Research Laboratories of Rockville, Md. Western blot kits from other firms are being used in research and may be approved by FDA for wider use.

Assays like these detect HIV antibodies by capturing them on antigen-coated surfaces and using a color reaction to visualize the antigen-antibody complex. The commercially available ELISAs, which are called first-generation tests, use disrupted whole-virus preparations as the antigen. These preparations, though made from purified virus, are contaminated with cellular debris that can attract non-HIV antibodies and thus produce false positives.

Second-generation tests

Scientists now are developing second-generation tests that use one or more genetically engineered HIV antigens instead. Because these preparations are purer, the number of false positives is reduced. Another benefit is that the test manufacturer avoids the hazard of working with live virus.

Some of these second-generation tests are said to be so reliable and easy to use that they may allow rapid HIV screening to expand into new markets, such as developing nations, physicians' offices, and possibly even the home market. For instance, at the University of California, Davis, a team of medical researchers has developed a dot enzyme immunoassay (dot EIA) on a small plastic card. The assay detects antibody to gp41, the glycoprotein that spans the outer membrane of HIV. In this assay, a drop of serum is exposed to recombinant gp41 on the card. If anti-gp41 antibody is present, it binds to the antigen. After detection reagents are applied, the bound antibody signals its presence by the appearance of a blue dot.

According to James R. Carlson, an assistant professor of pathology and internal medicine who leads the UC Davis team, the prototype of this dot EIA was more than 99% accurate in preliminary evaluations using blood sera from foreign and U.S. sources. These results compare favorably with ELISA and Western blot results, he says. Moreover, the assay takes about 30 minutes to perform, compared with two to four hours for the typical ELISA. It's also less expensive than the ELISA—about 25 cents versus $1.00 to $4.00 for ELISA kits.

A test like the dot EIA seems to be tailor-made for remote areas that lack highly skilled technicians or laboratory facilities, such as Africa. In some African cities, as many as 18% of the blood donors have been found to be HIV-infected, Carlson says, "yet donor blood isn't usually screened due to technical and economic constraints." The test is being developed for clinical use in a joint venture between Virotechnology Laboratories of Stockton, Calif., and Bio-Medican Corp. of Huntington Beach, Calif.

An even faster second-generation test has been produced by Cambridge BioScience, a biotechnology firm based in Worcester, Mass. The basis of this test is Recombigen, a novel recombinant antigen that attracts antibodies to both gp41 and gp120, the virus's envelope glycoprotein. "All of our data show that envelope antibody is the earliest and most important marker for detecting HIV antibody," says Rod N. Raynovich, vice president for business development at Cambridge BioScience.

The firm's test involves mixing a drop of blood serum with a drop of latex reagent on a plastic card. The reagent contains microscopic latex beads coated with Recombigen. If antibodies to the envelope proteins are present in the sample, the latex beads clump. The resulting clusters become visible in minutes as small white dots. If clumping has not occurred within five minutes, the sample is considered HIV-free.

In June, Thomas C. Quinn of the Immunoregulation lab of the National Institute of Allergy & Infectious Diseases (NIAID) reported at the 3rd International Conference on AIDS in Washington, D.C., that the Recombigen latex agglutination assay had been tested on sera from 2000 patients residing in Africa and the West Indies. Those tests indicated that the assay was accurate more than 99% of the time, and was more specific than a single ELISA. Quinn also said that the latex test was faster and simpler than the ELISA, and reliable enough that confirmatory tests might not be needed. Raynovich believes the Recombigen assay is better than the Western blot, and could replace it as a backup test.

Cambridge BioScience hopes to receive government approval to begin marketing the latex test in 1988 for use in physicians' offices and other low-volume, "decentralized" testing sites. The firm began conducting clinical trials of the test in September. Raynovich says the company plans to submit the results to FDA by the end of this year. In the meantime, FDA has allowed Cambridge BioScience to ship 50,000 test kits to Zaire, where the test is being used to screen for HIV-infected blood.

Murex Corp. of Norcross, Ga., also is developing a fast HIV screening test, but this one doesn't rely on a single recombinant protein. Rather, it uses seven natural HIV antigens that have been isolated by affinity purification using monoclonal antibodies, according to Gerald A. Bush, who has been leading the development effort. These antigens react with antibodies to the three major types of structural proteins in the virus—envelope, core, and replication-related proteins.

The reaction between these purified antigens and serum antibodies takes place inside a small, disposable, plastic cartridge. A positive reaction is indicated by the appearance, within 10 minutes, of a blue color in the cartridge's window.

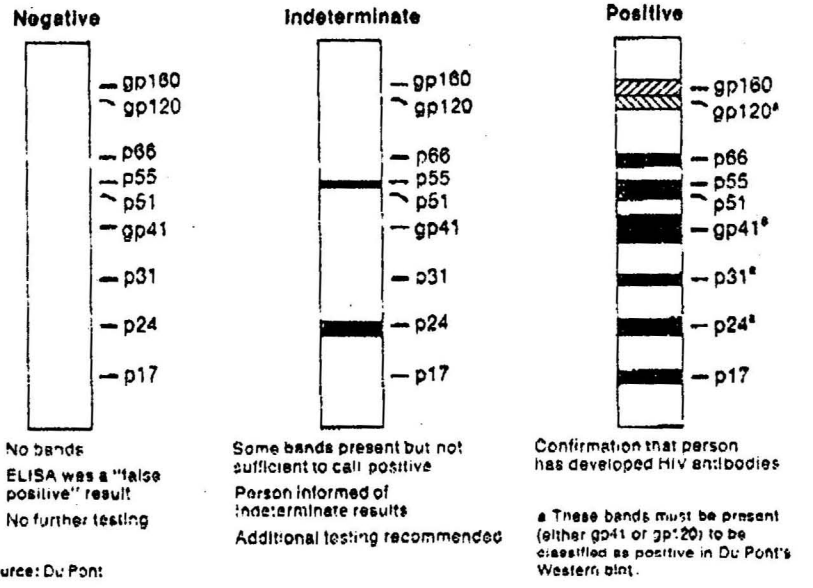
In early tests, the sensitivity and specificity of the Murex assay have been greater than 99%, says Bush. The assay actually picks up seropositive blood earlier than the ELISA, he notes. That might be because the purified antigens are so highly concentrated that they can detect lower levels of antibody, he explains. Bush says Murex expects

How ELISA and Western blot tests work

The screening of blood samples for antibodies to HIV (the AIDS virus) usually starts with an enzyme-linked immunosorbent assay, or ELISA. In this assay, blood serum is diluted and placed in microtiter wells or on beads that have been coated with HIV antigen (derived from disrupted virus that has been grown in cell culture). If the specimen contains antibodies (immunoglobulins), they will bind to the test kit's antigens. After an incubation period of about two hours, any unbound antibody is washed away. An enzyme-labeled antibody to human immunoglobulin is added, and this binds to any HIV antibodies that have become bound to antigen. The bead or well is again washed to remove any unbound antibody. A substrate is added and the enzyme bound to the antihuman antibody catalyzes a reaction that produces a color change. The optical density is measured spectrophotometrically and is directly related to the amount of HIV antibodies in the sample. The absorbance reading is interpreted by comparing it to positive and negative control samples containing known quantities of antibodies.

Samples that repeatedly test positive by ELISA are subjected to a different, more expensive procedure called the Western blot. This test identifies antibodies to the major HIV antigens. These antigens, derived from purified disrupted virus, are fractionated by size using polyacrylamide gel electrophoresis by the test's manufacturer. The fractionated viral proteins are then transferred onto nitrocellulose paper. A serum sample is applied to a strip of the paper. If antibodies to HIV are present, they will bind to the viral antigens. An enzyme-labeled antibody to human immunoglobulin is applied to the paper to bind to any HIV antibodies bound to antigen. An appropriate substrate is added and the enzyme catalyzes the color reaction. If HIV antibodies are present, a pattern of distinctive bands appears on the strip. The location of each band indicates reaction to a specific viral protein and is compared visually with a Western blot from a specimen in which antibodies to all of the HIV proteins are present. A positive result on this test almost always means the blood sample contains HIV antibodies.

Western blot readings have three possible interpretations



Source: Du Pont

to begin clinical trials of the assay by the first of the year, and the test to be licensed in 1988.

Home test for HIV?

A special feature of Carlson's dot EIA and the Cambridge BioScience and Murex rapid tests is that they work with whole blood as well as serum, according to sources involved in their development. That means they have the potential to be over-the-counter or home tests. But scientists and company officials discount this possibility, at least for now. They worry about the medical and ethical implications of AIDS testing in the home. Positive test results can devastate people, and any test could give a false positive, they say. "It's like giving somebody a death sentence," remarks one company spokesman.

Health care professionals agree that people need proper counseling to correctly interpret the results, plan confirmatory tests, and obtain medical and psychological therapy, if needed. "We believe a physician must be in the loop," says Raynovich. "We don't think FDA would approve a home test [for HIV] in the near term."

Similar thinking prevails at Du Pont, which is clinically evaluating an HIV antibody test the size of a matchbook. This test, like its potential competition, uses a color change to give the same yes-or-no information as ELISA, only much quicker (in five minutes). Du Pont spokesman Mike Ricciuto says the firm hopes to begin marketing the matchbook test in Africa later this year. In the future, he says, Du Pont might seek FDA approval to sell the test to hospitals and physicians in the U.S. "It would probably never be marketed as a home test," he adds.

Because any of these screening tests could produce false positives, most scientists believe that there's still a need for confirmatory assays such as the Western blot. This test, sometimes called the immunoblot, provides more information than the standard ELISA because it exposes a serum sample to a strip of paper containing all the key HIV antigens separated according to their molecular weight. Thus, the identity and relative abundance of specific antibodies in the sample can be

visualized on the strip according to the specific antibody-antigen reactions that occur.

Problems with Western blot

Nevertheless, indeterminate and false positive results can arise on the Western blot because the method is not standardized. A 1986 Institute of Medicine report on AIDS noted that "There has been considerable variation in what different laboratories have interpreted as positive in Western blots." In the past, some testing labs have considered antibody to the core protein p24 as sufficient for a positive result. The Institute of Medicine report states that this is an equivocal result. The National Institutes of Health requires antibody to p24 and to the transmembrane protein gp41. Du Pont says a positive result must include p24, p31 (a replication-related protein), and either gp41 or gp120.

Improvements in the Western blot have helped to some extent. Earlier versions of this assay were deficient in detecting antibodies to gp120 because this key protein was selectively lost during the manufacturing process. The newer versions of the test, such as Du Pont's, offer enhanced detection of antibodies to gp120 and its precursor protein gp160, making it easier to classify "some potentially ambiguous sera," says Stanley H. Weiss of the National Cancer Institute's environmental epidemiology branch.

Even so, the interpretation of Western blot reactions can still be problematic. Weiss notes, for example, that some persons who had "unusual Western blot reactivity patterns" or showed no evidence of HIV antibodies were found to be HIV-infected by virus culture. As a precaution, blood banks currently reject donations that repeatedly test positive by ELISA, even if they test negative in the Western blot. Many of these ELISA results certainly are false positives, and the blood that is thrown out is wasted.

In an effort to clear up the uncertainty in some Western blot readings, scientists at Abbott Labs have devised an immunoassay that is complementary to the Western blot. This assay, Envacor, actually consists of two tests: One detects antibody to an envelope protein (gp41) and the other detects antibody to a core protein (p24).

Envacor may be useful as a diagnostic tool because the two antibody types provide different information. "Envelope antibodies are present in virtually everybody who's infected," says Jean-Pierre Allain, who manages Abbott's research on AIDS diagnostics. They are "an extremely stable and reliable marker for HIV infection" throughout the course of the disease, he adds. Antibodies against p24, by contrast, are not always present and their level changes depending on the stage of infection. About 85 to 90% of infected asymptomatic persons have anti-p24 antibodies. But as these individuals become sicker, anti-p24 levels drop lower and lower, eventually becoming undetectable. Thus, Envacor might be useful in charting and predicting the immunological course of the disease, says Carlson, who directs the AIDS Virus Diagnostic Laboratory at UC Davis.

The Abbott test offers a number of advantages over the Western blot, according to Allain. Envacor is easier to perform because it's in an ELISA format. It gives a numeric readout of results, thus avoiding the subjective interpretations required by Western blot. It's also quicker, although it requires an overnight incubation step like the Western blot.

Allain: immune system collapse

University of California's Carlson holds the dot EIA test for HIV

On average, the two tests are of similar sensitivity. But Envacor's specificity is very high—99.9%. This means that, statistically, 999 out of 1000 HIV-negative blood samples will be unreactive in the test. Only one in 1000 is likely to turn up as a false positive. In Europe, where Envacor has been in use for more than a year, Abbott recommends that it be used in conjunction with the Western blot.

Tests for viral antigen

The variability of the immune system's antibody response to HIV proteins in different people has led researchers to develop a third generation of AIDS tests, which detect the virus or its fragments directly. This approach promises to catch some of the false negative results that crop up in antibody tests.

False negatives occur in persons who haven't yet developed detectable levels of HIV antibodies. Typically, antibodies aren't detectable in blood until six to 10 weeks after infection, according to Jaap Goudsmit, head of the retrovirus laboratory at the University of Amsterdam. A recent study of homosexual men in Finland has found that some HIV-infected persons don't develop antibodies for a year or more. And some HIV carriers may never mount an antibody response at all. Even so, these individuals are still considered infectious, and their blood may contain detectable levels of antigens.

Obviously, one way to pick up infected blood earlier than is possible with antibody tests would be to look for viral antigens. Several companies are developing such antigen tests for marketing. Abbott and Du Pont unveiled their entries in June at the 3rd International Conference on AIDS. Although their antigen tests aren't yet available commercially in the U.S., they have been used as research tools for at least a year.

The Abbott and Du Pont antigen tests are both enzyme immunoassays that directly detect the core protein p24, which is shed by the virus. The Abbott test, for instance, uses purified, naturally occurring antibodies from an infected individual. These are bound to a solid phase and are allowed to capture whatever antigen may be in the sample. The visualization chemistry is similar to that used in other enzyme immunoassays.

Researchers stress that such direct-virus tests will complement, not replace, antibody tests. That's because the levels of antigens and antibodies change with time, and looking at only one or the other can give a misleading result. For example, if a person is producing antibody, then antigen in the blood will be masked to some extent by the binding of the two, Du Pont's Ricciuto explains. In this instance, antigen may not be detected at all, although an antibody assay would correctly signal HIV infection. Hence, it would be necessary to screen blood for both antibody and antigen, he says.

The rationale for adding the antigen test to blood screening programs is to eliminate donations of infected but antibody-negative blood. However, no one really knows whether this test will be able to pick up infected blood early enough to warrant the additional cost, says Ricciuto. According to John P. Phair, professor of medicine at Northwestern University Medical School in Chicago, since blood screening for HIV was instituted in 1985, scientists have documented one case in which HIV was transmitted by transfusing antibody-negative blood. Phair noted in a recent editorial in the *Journal of the American Medical Association* that blood banks would have to spend at least $5.00 to $7.00 per unit of blood to test for antigen. The added expense is not justified at this time, he concluded.

The antigen test might be more important as a way to diagnose AIDS earlier than is now possible. And

Diagnostic tests being developed for other retroviruses

In the U.S., as far as anyone knows, all cases of AIDS have been caused by the same retrovirus—a human immunodeficiency virus (HIV-1).

In Africa, the situation is more complicated. HIV-1 is widespread in Zaire and other central African nations. But a closely related human retrovirus, HIV-2, is endemic in Senegal and several other countries in Western Africa. HIV-2 appears to cause an AIDS-like disease. Both HIV-1 and HIV-2 are highly prevalent in at least two West African countries. Furthermore, Africa also is the home of simian immunodeficiency virus (SIV), which appears to be more closely related to HIV-2 than to HIV-1. SIV doesn't infect humans, but it does cause an AIDS-like disease in some monkeys.

The prevalence of infections caused by these viruses in Africa appears "catastrophic," according to a research team that includes Erling Norrby of the Karolinska Institute in Stockholm and Richard Lerner of the Research Institute of Scripps Clinic in La Jolla, Calif. They say there is "an emergency need" for simple, inexpensive, sensitive, and specific tests that can identify antibodies to these retroviruses, and distinguish between them.

Several groups have been working to develop such immunoassays, and at least two—Norrby's and a different group headed by John W. Gnann Jr. of Scripps Clinic—recently reported success. Both groups have based their assays on synthetic peptide antigens derived from the transmembrane glycoproteins of the different viruses [*Nature*, **329**, 248 (1987); *Science*, **237**, 1346 (1987)]. These antigens were incorporated into the standard laboratory screening format known as ELISA. Besides pointing the way toward better diagnosis of AIDS infections, early results from this research offer hope that it will be possible to solve what Gnann's team calls "the epidemiologic puzzle of AIDS in Africa."

Researchers also have been testing blood from American donors to check if HIV-2 has spread to the U.S. So far, no evidence of this has turned up. For now, it seems, HIV-2 tests will remain for investigational use only.

Scientists and health officials are more concerned that another insidious retrovirus may be spreading through the U.S. blood supply—HTLV-1, or human T-cell lymphotropic virus, type 1. This virus causes adult T-cell leukemia in about 1% of those infected. Like AIDS, this cancer is fatal and may not appear until years after infection. HTLV-1 is transmitted in the same way as HIV—through sexual contact, exchange of blood by hypodermic syringes, and transfusion of infected blood or blood products. Evidence indicates that many drug addicts in the U.S. have already been infected with HTLV-1, which is also implicated in other diseases.

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Nevertheless, many companies already see the writing on the wall. At present, all blood donations in the U.S. are screened for HIV antibodies and "we believe it is only a matter of time" before blood screening for HTLV-1 also will be required, says John R. Zeman, vice president and general manager of Eastman Kodak's clinical products division. Kodak plans to market a test manufactured by Cellular Products that can simultaneously detect HIV and HTLV-1 antibodies. Du Pont, too, is already gearing up to produce millions of HTLV-1 antibody test kits a year.

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Certainly, the antigen test is expected to be an essential tool for monitoring the efficacy of experimental anti-HIV drugs by measuring antigen levels after drug administration. According to Paul A. Volberding, who directs AIDS activities at San Francisco General Hospital, the Abbott antigen test helped researchers determine that the drug zidovudine (popularly known as AZT) retards the virus's replication in the body. This was seen in declining levels of p24, which is used as a marker of viral burden.

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Researchers are applying nucleic acid probes to accomplish this mission. Such a probe consists of a synthetic sequence of nucleotides—either RNA or DNA—that is complementary to the target viral sequence. When the probe encounters its target, the two strands hybridize, or join together to form a double-stranded segment. Because the probe sequence is tagged with a radioactive or nonradioactive label, the site of the target sequence can be located.

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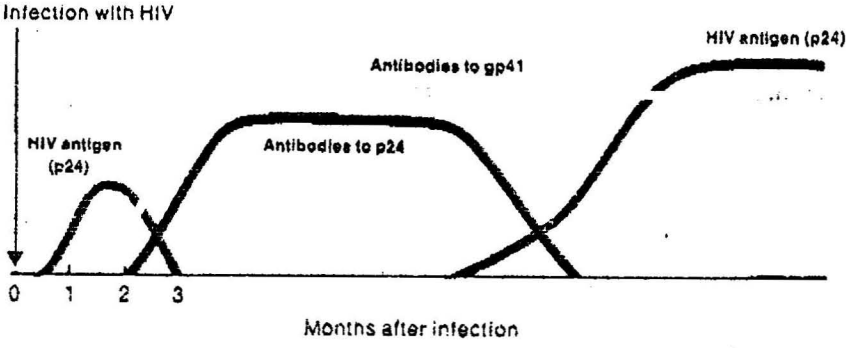
Although researchers now can test for antibodies, antigens, and even bits of viral genes, they still don't have a clear idea of what the test results mean in terms of the patient's clinical condition and prognosis. Perhaps, one diagnostics analyst tells C&EN, that all-important connection will be made once scientists have in place a battery of assays that can be run routinely on AIDS patients. ◻

Core antigen, antibody levels vary as disease progresses



A hypothetical patient's profile shows the inverse relationship between two key HIV markers—the core antigen p24 and antibodies raised against it. p24, which appears in blood soon after infection, is neutralized by a rising tide of anti-p24 antibodies. As long as the level of these antibodies remains high, the patient's condition is stable. But months or years later, when the virus starts replicating again, its stepped-up production of p24 overwhelms the immune defenses by complexing all the available anti-p24 antibodies. By contrast, the level of antibody to the transmembrane glycoprotein gp41 remains fairly constant

Source: Abbott Laboratories

The Washington Post

FEBRUARY 22, 1988

Washington Business

Home Test Kits For AIDS Are Being Readied

Firms Accused of Trying To Profit From Public Fear

By Malcolm Gladwell
Washington Post Staff Writer

Mark Siljander and Stanley Lewis call themselves entrepreneurs providing a valuable service. Their critics accuse the two local businessmen of attempting to cash in on the public anxieties over the AIDS epidemic.

Over the next few months, the Food and Drug Administration will decide which side is right.

At issue are the separate plans of Siljander, a onetime congressman from Michigan who is now based in Reston, and Lewis, a Baltimore psychologist, to sell home AIDS test kits in drug stores that will allow consumers to test themselves for AIDS without going to a clinic.

The two are part of a national trend that has seen private firms increasingly attempt to move into the AIDS-testing business, adding a for-profit dimension to a service that until now has been dominated almost entirely by publicly funded, nonprofit health clinics.

But the proposals also have raised a host of ethical and regulatory questions, and sparked vehement opposition from many public health officials who question whether private firms—especially those offering home testing kits—will provide adequate service, safety and, in particular, counseling to clients.

While both Siljander and Lewis predict they will be selling the home testing kits by the end of this year, the final decision rests with the FDA, which has jurisdiction over all medical testing devices and services. The agency is now considering the matter, and has given no indication of when it will decide.

Siljander, who had the backing of the Moral Majority when he ran for Congress and a taste of controversy during his three terms in Congress, formed the American Institute for the Detection of AIDS last year with two Chicago doctors. Buyers of Proteck, as his kit will be known, get a lancet to draw their own blood, a special box to send the sample through the mail to an licensed lab, and a 900 number to call a week later to get the result.

"We're looking for people who have been promiscuous but who are embarrassed to go to a doctor," Siljander said. "There are millions of people out there who don't want to go to a clinic."

The government has estimated the number of Americans potentially at risk for AIDS who

See TESTING, page 24

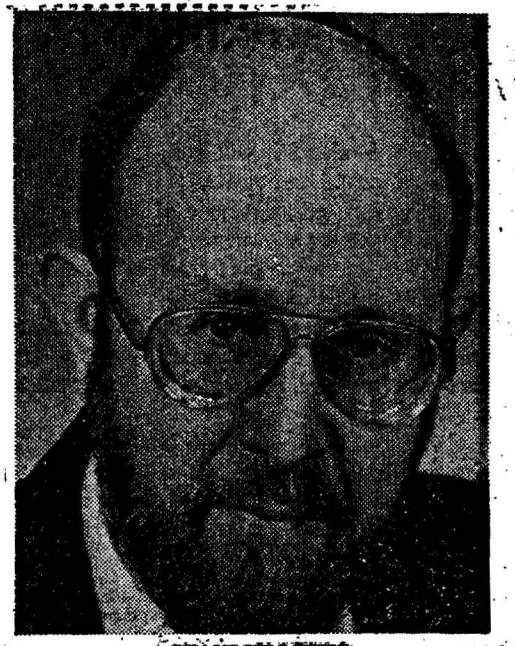
TESTING, from page 1

haven't yet been tested at between 30 million and 40 million.

Lewis, meanwhile, bought Peace of Mind Inc., a Nevada-based manufacturer of rubber "bumpers" for ladders, last summer and has turned it into a multifaceted high-tech research outfit with an eye to AIDS "marketing strategies." The company plans to market a similar home blood-collection and testing kit, saying that it has lined up "upscale retail locations" to sell its product. "We want to market this test to middle America," Lewis said.

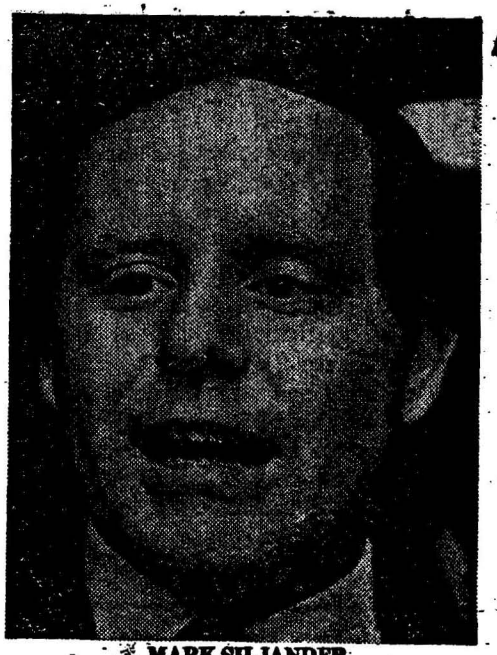
Siljander's and Lewis' companies are two of several firms that have asked the FDA for permission to enter the AIDS home testing market. But there are other local entrepreneurs in other facets of the AIDS-testing business. Two Maryland businessmen, Wayne Simmons and Mark Regan, for example, opened a clinic in Greenbelt last summer offering confidential AIDS testing for a flat fee of $55, and have just completed a private placement that could see their company, Blood Check Inc., open seven additional facilities in and around Washington and Baltimore over the next few months.

Blood-Check and services like it, however, are essentially expanded commercial versions of the private testing that many doctors have offered in their offices since the beginning of the AIDS epidemic. Home collection kits, on the other hand, place AIDS testing for the first time outside the medical community, a fact that has made them the most controversial of the for-profit testing alternatives being put forth by private companies.



STANLEY LEWIS
... wants "to market this test to middle America"

REP. RON WYDEN
... field is "ripe for fraud and manipulation"



MARK SILJANDER
... seeks people "embarrassed to go to a doctor"

"This is a brand new field. There's a chance to make enormous sums of money, but we've got essentially no regulation," said Rep. Ron Wyden (D-Ore.), a member of the House health and environment subcommittee. "It's an area that's ripe for fraud and manipulation. Home kits go right to the heart of people's fear."

Wyden and two other congressmen wrote to FDA Commissioner Frank Young last October asking the agency to "take proper precautions to insure the safety and efficacy of the test kits." The letter focused not so much on the question of accuracy—the testing procedures and laboratories to be used by home kit manufacturers are the same as those used by health clinics—but rather on the safety of sending possibly infected blood samples through the mail, as well as the question that, for many, is the critical issue surrounding home testing: whether face-to-face counseling services are made available to consumers who test positive for the disease.

At present, publicly funded AIDS clinics are required by health officials to provide counseling before and after testing to explain the test and to help clients prepare emotionally for the results.

"Everyone recognizes that the value of testing is directly related to the quality of the counseling associated with it," said Jeff Levy, executive director of the National Gay and Lesbian Task Force, one of a number of groups that have strongly endorsed the government's counseling requirement.

Because the proposed home testing services rely so heavily on mail and phone contact with kit buyers, critics have questioned whether they can provide an adequate level of counseling. Few suggest that the FDA will go so far as to ban home kits on these grounds—indeed, many experts believe that home testing, in one form or another, is probably inevitable.

What the FDA is trying to do, however, is to decide what restrictions and conditions to place on the sale of the kits: how to balance the convenience and accessibility offered by home collection against the dangers inherent in a test environment that is outside of a medical setting.

The entrepreneurs hoping to sell the kits said they plan to offer counseling to their customers. Siljander said his Proteck kit, for example, will have counseling instructions inside the package, trained counselors staffing the 900-number phone lines "with answers to the 18 or 20 most often asked questions," plus a list of counseling services around the country to which testees can be referred.

Is that enough? Siljander thinks so, but many public health officials disagree. "Testing by itself is a waste of time without high-quality education," said Peter Hawley, medical director at the Whitman-Walker Clinic in Washington, which handles much of the region's AIDS testing. "It's not good enough to hand people a lot of brochures. Some people won't read them. Some people have mixed emotions about AIDS, some of which has to do with denial. We have people who come out of these sessions thinking that if they test negative they must be immune to AIDS. All sorts of misinformation gets communicated."

"If you ask anyone who has done this kind of work in testing centers, you will find out that people are in need of direct hands-on counseling," Levy said. "Calling hot lines doesn't do the trick."

Peace of Mind's proposal goes a step further, asking test applicants to pick up their results from special offices the firm hopes to open in 150 cities. Rather than have trained counselors on staff, the company plans to offer clients who test positively for the AIDS virus an interactive computerized video counseling session.

"Our concern was that the people currently doing counseling are doing a poor job," Lewis said. "We wanted a system set up in an entirely standardized fashion by us."

Lewis said many existing counseling efforts fail because recipients don't understand what they are told. So Lewis' computerized system requires viewers to answer questions at each stage of the five-part session.

"They've got to understand what's going on," Lewis said. "Otherwise they can't get into the next section."

But counseling by video and computer doesn't satisfy some health officials. "The post-test counseling has to be done one-to-one. It can't be done any other way," Hawley said. "Interactive video is not capable of judging the emotional state of the patient. . . . I'd hate to have a patient find out they're positive, and then walk out and kill themselves."

The AIDS-testing entrepreneurs respond to these charges by stressing what they see as the failure of publicly funded testing efforts to reach people outside the highest risk groups.

"A lot of people don't want to go to these clinics. They're often not in the best parts of town. They don't offer much privacy," said David Pivar, president of California's Discrete Medical Testing Inc., another firm hoping to enter the home collection business. "The easier the accessibility of the testing, the more people who think they are at risk will take it."

Blood-Check's Wayne Simmons believes that pre- and post-test counseling required by public clinics may be a big reason why many Americans decline to be tested for AIDS.

"We never send anyone out of the facility if they need help," he said. "But the choice is left to the individual. We see anything mandatory about AIDS testing as a deterrent." Simmons said market research by his firm shows that 77 percent of respondents indicated that they wanted counseling to be available but not compulsory.

By doing away with counseling requirements and putting a kit in neighborhood drug stores, testing firms think they can attract the broadest possible market.

"We happen to believe the major marketplace for this kind of product is the millions of businessmen, government employees who travel, who have committed an indiscretion and are not about to tell their wives or to go anywhere and have a test," Peace of Mind's Lewis told an FDA advisory committee meeting in December. "We have not done any market surveys. Perhaps we could start with this building and survey the government employees to see how many of them are really worried about that."

Siljander hopes to enlist celebrities and public figures for an extensive "It's Best to Know" campaign to encourage Americans to be tested.

Home testers also intend to price their services aggressively. The charge for a preliminary AIDS test conducted by a private physician can run in excess of $75—a reflection of the high prices private laboratories charge for processing the samples. Siljander expects to sell his kit in drug stores for $9.95, plus a $20 or $25 surcharge to get the test processed. Lewis told the FDA he could go even lower: to $18. "I promise you, at $18 there is a very healthy margin," he said.

But few believe that competitive pricing alone will end the controversy over home testing.

"Let's talk candidly for a moment. This type of disease brings out every kook from the woodwork," said Siljander. "Some of these guys have little or no credibility."

"I can't tell anybody not to deal with these people," Reed Tuckson, D.C. commissioner of public health, said of the private AIDS testers. "But I would above all hope that any person involving themselves with a private firm would make very sure that their anxiety is not being exploited by unscrupulous persons. Citizens ought to be prepared and aware that there are people who may try to exploit them."

New York Times 4/10/88

Spending Strategies Awaited

In AIDS Research, Money Is Just the Start

By ROBERT PEAR

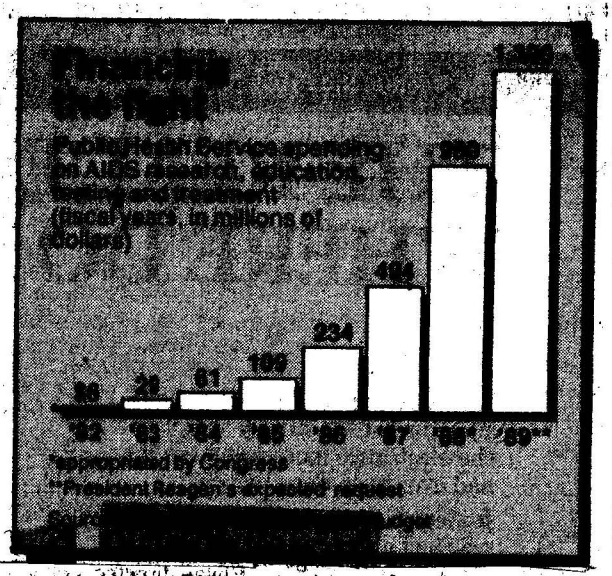
WASHINGTON

SEVEN years into an epidemic that is expected to kill more than 179,000 Americans by the end of 1991, Federal financing to fight AIDS is approaching the amount spent in the war on cancer. Budget documents show that President Reagan plans to ask Congress for $1.3 billion in the next fiscal year to help the Public Health Service combat the epidemic, an increase of 37 percent over appropriations for the current year. By way of comparison, the National Cancer Institute's 1988 budget is $1.5 billion.

As AIDS spending continues to grow dramatically, from $234 million in the fiscal year 1986 to $494 million in 1987 and $950 million this year, Federal officials are confronted with some difficult questions. How can the Government nudge research in desired directions while allowing individual investigators the freedom and creativity that often produce the most important scientific discoveries? How much of the money should be spent in Government laboratories? How much at universities, medical schools and hospitals?

"The best science comes from individual scientific creativity," said Dr. Anthony S. Fauci, coordinator of AIDS research at the National Institutes of Health. "We have to guide and coax in the right direction, but we cannot push too hard. We are against the idea of an AIDS czar or an approach like the Manhattan Project. That may be the way to get an atomic bomb, but that is not how you get the best science."

When the new figure for AIDS spending was disclosed last week, scientists said that Federal outlays appeared, after much delay, to be approaching the levels they regarded as necessary. A report by the National Academy of Sciences in 1986 said that by 1990 the Government should provide $1 billion a year for research on AIDS and should make significant contributions to the additional $1 billion a year required for education and public health measures. The President's 1989 budget re-



quest includes $123 million for research into the causes of AIDS, $328 million for development and evaluation of drugs and vaccines, $387 million for prevention activities and $24 million that will probably be used for a new laboratory for the Food and Drug Administration.

As the amount of money increases, competition becomes more intense. In the quest for financial support, scientists studying molecular biology and the structure of the AIDS virus are, in a sense, competing with epidemiologists who do field work to track the spread of the virus. Both types of work are essential to understand and disrupt the virus, which may become one of the most common causes of death, after heart disease and cancer, in the 1990's.

Unanswered Questions

Dr. Charles C. J. Carpenter of Brown University, chairman of a group of consultants who advised the N.I.H. on future directions for AIDS research, said scientists had made progress in both the molecular biology and the epidemiology of AIDS, identifying the genetic characteristics of the virus and the routes of transmission. But, he said, much work is needed to learn which cells become infected and what changes occur immediately after exposure to the virus. If scientists knew more about the mechanisms by which the virus causes disease, it would help them in trying to devise vaccines and therapeutic drugs.

Dr. Fauci said recently about 10 percent to 15 percent of the N.I.H. funds were spent for research by Federal scientists working in Government laboratories and clinics. The remainder, he said, goes to private researchers for projects initiated either by the investigators themselves or by the Government. The Government, for example, has signed contracts with university medical centers to perform clinical trials of experimental drugs in people infected with the AIDS virus.

Researchers who design their own projects usually receive grants, which are less restrictive than contracts. In evaluating applications for grants, the N.I.H. uses an elaborate system of peer review developed over the last 40 years. At the moment, officials said, the competition among scientists working on AIDS is not as great as that among scientists working in some other areas of biomedical research, such as metabolic causes of heart disease. But as AIDS researchers gain experience, their applications are receiving higher scores in the peer review process, indicating an increase in their technical and scientific merit, Dr. Fauci said. "We are funding most of the applications that are worthy of being funded," he said.

The report by the National Academy of Sciences warned that an overcommitment to centrally planned studies could have "extremely deleterious consequences" if it excluded "creative scientific input from researchers outside of the N.I.H." Federal officials said that investigator-initiated projects accounted for a growing share of the total because more and more experienced researchers were being attracted to the study of AIDS.

Dr. Carpenter's advisory panel warned that the Government must not shortchange other biomedical research to pay for work on AIDS. Some of the research that appears unrelated to the disease "may, in fact, provide important basic information in areas of immunology, virology and molecular biochemistry that will be critical to the resolution of the AIDS epidemic," the panel said.

It noted that the initial progress in AIDS research resulted, in large part, from money spent on basic research into viral causes of cancer over the last two decades.

On January 20, 1988 Fortune Magazine and Allstate Insurance Co. released a comprehensive study on executive attitudes toward AIDS. I found the study both alarming and instructive. The answers from 623 randomly selected American businesses rangeing from big to small to a 12 page questionnaire found that American business in general does not have policies that are supportive of AIDS infected employees.

I am sure that if a similar study was conducted in other countries, the result would be the same. I am certain of this since, the study further concluded that as the incidence of the disease increases in the workplace, the incidence of supportive attitudes, behaviors and policies also increases. Since the incident level in other countries is similar to that of the U.S. one can conclude that businesses in these other lands are equally unsupportive.

On the brighter side, if anything can be bright about AIDS, is the study's conclusion that the more knowledgeable company executives become about AIDS the more supportive are attitudes and company policy. In fact, companies that have had employees with later stages of AIDS are more likely to have knowledge that results in a supportive written policy. But from my perspective, waiting for an increased incidence of AIDS cases before taking actions is not in the business world's best interest. I say this in the face of the study's finding that appeals from others outside the confines of the company have not been effective in getting the company involved with the AIDS problem.

The PHS has estimated that between 1 to 1.5 million Americans have been infected by AIDS. Given that a vaccine will be many years in development it seems clear we must use all our current knowledge to both decrease the rate of infection and care for those that are infected. This makes sense to business not only for humanitarian purposes but economically. The study reveals that business executives believe the cost of treating employees with AIDS lies primarily with insurance companies and secondarily with the employees with AIDS. At the same time they recognize that while their medical insurance premiums have not yet been affected, they expect a substantial increase within the next 5 years. Further, as the cost of the medical insurance premiums increases, most executives believe that all employees will be asked to contribute more of the cost of their medical benefits and that benefits will be reduced.

Given the prospect of higher costs to the employer or lower employee morale or both, it seems sensible to me that the business world needs to use all current knowledge to prevent AIDS through employee education and policy direction.

As I noted, the Fortune study has been instructive. I must admit that Maxwell Communications Corporation has not pursued the course I've suggested here for others. In order to match my corporate words with corporate deeds I have directed my staff to contact Allstate Insurance Company in regard to the AIDS handbook produced in conjunction with their study and to further solicit their advice on the development of a written policy on AIDS for Maxwell Communications Corporation. I believe our corporation cannot afford to be within the ranks of the four of every five companies surveyed not having such a policy.

Opening the Channel for New AIDS Drugs

FDA Needs More Money and Personnel to Speed Approvals, Panel Asserts

By Sally Squires
Washington Post Staff Writer

Major changes are needed in the country's drug approval process to meet the demands of the growing population of AIDS patients, a president's panel concluded last week.

According to retired Admiral James D. Watkins, the chairman of the Presidential Commission on the Human Immunodeficiency Virus Epidemic, there is a pressing need to speed up the drug approval process so that more AIDS patients can be helped. Watkins cited some serious flaws in the current system that are slowing the emergence of new drugs to treat AIDS.

His report, which covered a broad range of issues including drug development, was endorsed by the full panel yesterday and will go to President Reagan later this week.

Among the numerous problems Watkins cited in the 60-page report is the Food and Drug Administration's need for more money, additional personnel and better facilities to handle the increase in experimental drugs that are being developed.

The present FDA facilities "are inadequate to deal with the pharmaceuticals that are coming downstream from the National Institutes of Health," Watkins said. If the FDA uses its present resources to concentrate on AIDS, he said, other diseases will suffer.

So far, the FDA has received some 179 applications for approval to test 120 new AIDS drugs, diagnostics and vaccines, according to Dr. Frank E. Young, FDA Commissioner.

Yet this large number of applications threatens to overwhelm the FDA drug approval process and stymie the development of not just AIDS drugs, but of all pharmaceuticals in the United States, Watkins said.

It takes most drugs an average of seven to 10 years to go from development to final FDA approval. In contrast, the AIDS drug AZT (Azidodeoxythymidine) went from the laboratory bench to physician's office in just two years—in part, because the FDA "expended eight man years of effort at a cost of $600,000," Young told the commission on Feb. 19.

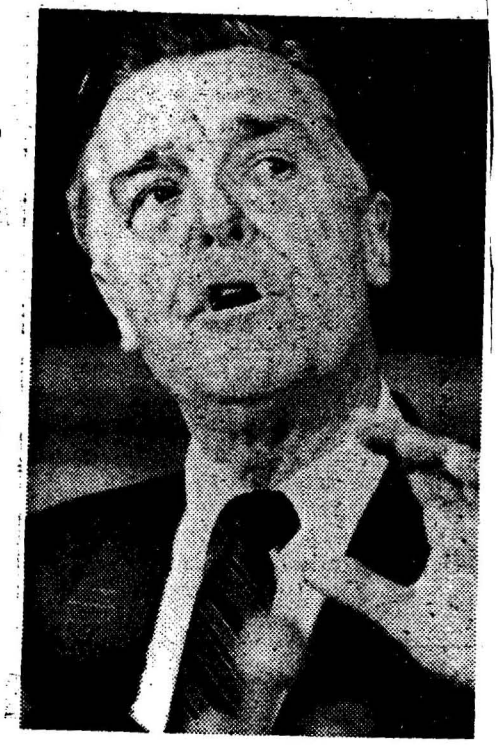
It took the FDA 2½ months to review the scientific data on AZT and approve the drug for use in AIDS patients.

But since the FDA has neither the money nor the personnel to continue reviewing drugs at that pace, there is concern that the entire drug approval system will bog down.

"We are very concerned that we don't have a system to move these drugs rapidly into clinical trials. There needs to be a significant enhancement of that process," said Watkins.

"We are very concerned that we don't have a system to move these drugs rapidly into clinical trials. There needs to be a significant enhancement of that process."

— Adm. James D. Watkins
AIDS Commission Chairman



Wash. Post | Health
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Without major revisions in the drug-approval process, the FDA "is going to be in very serious trouble very soon," Watkins said at a breakfast meeting last week. "The FDA is kind of an orphan agency in Washington. We're picking up [FDA Administrator] Dr. Young's advocacy."

One of the key recommendations of the Presidential Commission's report is a plan to eliminate placebo-drug trials for AIDS patients.

Placebos are sugar pills. In clinical trials of new drugs, researchers give one group of patients the experimental drug. Another group of patients receives the placebo. Neither the patients nor the researchers know until after the testing is completed (and a code is broken) which individuals received the drug and which received the placebo.

Using a placebo allows researchers to eliminate the "placebo effect." Sometimes when people think that they are taking a drug, they get better—not because the drug makes them improve but because they believe they will get better.

"We don't need any more placebo-controlled drug testing for AIDS patients," Watkins said, who noted that these patients are so desperately ill that it is unfair to give them sugar pills. Scientific investigation of new drugs can be served instead, he said, by involving people who test positive for exposure to the virus or those who have the pre-AIDS condition known as ARC—AIDS Related Complex—in placebo drug trials.

The reaction from the scientific community to eliminating placebo drug trials has been mixed. "I don't see any need for any requirement for a placebo-controlled trial in people with [full-blown] AIDS," said Dr. Samuel Broder, associate director of the clinical oncology program at the National Cancer Institute and one of the researchers who first proposed AZT for use against AIDS. "There are certain limits to what you can do when you are dealing with human beings who have life-threatening illness. Two years ago, the placebo-controlled trial method was absolutely essential. I do not believe that it is now."

But others fear eliminating placebo-controlled trials for AIDS patients might set a difficult precedent for other diseases. Robert Allnutt, vice president of the Pharmaceutical Manufacturers Association, a drug-industry trade association, said that placebo-controlled trials "are something that we have to look at very carefully." Eliminating placebo-controlled trials for AIDS patients "strikes me as being too broad a statement. But we will have to have our experts look at it."

The AIDS commission also called for:

- Immediate funding to help design drug studies in underserved populations with AIDS, such as women, children, minorities, intravenous drug users and people who are HIV positive but do not yet have the illness.
- Increased access to a greater variety of experimental treatments for people infected with the AIDS virus.
- Stepped-up search for a proper animal model for vaccine development against the AIDS-causing HIV virus.
- Wider availability to the scientific community of the pieces of the AIDS virus so that drug research can progress more rapidly.
- Adoption of an international standard so that the FDA can utilize high quality drug trial results from other countries.
- Doubling of the number of reviewers of HIV-related products at the FDA.
- The addition of $25 million in the FY 1989 budget for an additional FDA building to house new employees involved in AIDS research.
- Early release of new drugs, which can then be closely monitored once they are on the market.

All in all, the reaction of the drug industry to the Presidential Commission's recommendations has been generally favorable. "Admiral Watkins is calling for more resources to speed the approval of AIDS drugs, and coincidentally that would speed the approval process of all drugs," said PMA's Allnutt. "We certainly agree with that."

Meanwhile, NCI's Broder reported yesterday at an FDA seminar that he and his colleagues have begun testing a new AIDS drug called DDA (for di-deoxyadenosine) in AIDS patients.

DDA "is much less toxic to the bone marrow [than AZT]," Broder said. It may be that as in treating leukemia, he said, doctors will one day treat AIDS with several drugs, perhaps by alternating administration from week to week.

In studies of a combined drug approach, alternating AZT with another drug known as di-deoxycytidine, five of 15 AIDS patients have survived beyond six months. "One patient has gone beyond 36 weeks," Broder reported.

Contrary to the mood of pessimism about finding effective therapies for AIDS that dominated researchers several years ago, Broder said, "we now face a very large menu of drugs that have the activity and attack the AIDS virus at multiple steps in the life cycle.

"Now the question becomes what drug do you investigate and what priority do you give it? There is a real problem of how to prioritize drugs. It is an interesting challenge but not one that most of us predicted we would be facing."

While there is no question that AZT can prolong life for many of those with AIDS, Broder said, "I don't believe that it is a cure . . . This is a first step."

What research is indicating, Broder said, is that "we are going in the right direction." Studies have shown that AIDS "can be treated," he said. "That was uniformly not accepted in 1984."

There also seems to be one positive benefit of the deadly AIDS epidemic that has already emerged in the Presidential Commission Chairman's report: The disease is pinpointing some major flaws in the current health care system.

AIDS research is likely to have many "benefits to Americans who suffer from cancer, viral and immune-related diseases, which collectively kill an estimated 650,000 Americans each year," according to the chairman's report.

"AIDS may be the real catalyst for getting our attention [on medical problems] across the board," Watkins said. ". . . We have exposed significant health care problems in the nation." ■

AIDS DRUGS IN DEVELOPMENT

Anti-Virals

Drugs that directly attack HIV, the virus that causes AIDS, or other viruses

DRUG NAME (Generic name)	MANUFACTURER	INDICATION	DEVELOPMENT STATUS
AL-721	Ethigen (Los Angeles, Calif.)	ARC, persistent generalized lymphadenopathy (PGL)	IND approved Phase II
Betaseron (interferon beta)	Triton Biosciences (Shell Oil) (Alameda, Calif.)	AIDS, Kaposi's sarcoma, ARC	IND approved Phase I/II
Cytovene (ganciclovir)	Syntex (Palo Alto, Calif.)	CMV	NDA Pending (Orphan drug)
DDC (dideoxycytidine)	Hoffmann-La Roche (Nutley, N.J.)	AIDS, ARC	IND approved Phase I/II
(dextran sulfate; UA001)	Ueno Fine Chem. Industry (Osaka, Japan)	AIDS, ARC	IND approved Phase I
Foscarnet (trisodium phosphonoformate)	Astra Clinical Research (Hopkinton, Mass.)	HIV infection, CMV retinitis	IND approved Phase I/II
HPA-23	Rhone-Poulenc Sante (Monmouth Junction, N.J.)	HIV infection	IND approved Phase I
Ornidyl (eflornithine)	Merrell Dow (Cincinnati, Ohio)	Pneumocystis carinii pneumonia (PCP)	NDA pending (Orphan Drug)
Peptide T (octapeptide sequence)	Peninsula Labs (Belmont, Calif.)	AIDS	IND approved Phase I
Reticlose (nucleophosphoprotein)	Advcanced Viral Research (Miami, Fla.)	AIDS, ARC	IND submitted
Retrovir (zidovudine; AZT)	Burroughs Wellcome (Research Triangle Park, N.C.)	AIDS, advanced ARC	NDA approved
		Pediatric AIDS, Kaposi's sarcoma, asymptomatic HIV infection, less severe HIV, neurological involvement, in combination with other therapies	IND approved Phase I/II
Rifabutin (ansamycin LM 427)	Adria Labs (Dublin, Ohio)	ARC	IND approved Phase II
(trimetrexate)	Warner-Lambert (Morris Plains, N.J.)	PCP	IND approved Phase III
Virazole (ribavirin)	Viratek/ICN (Costa Mesa, Calif.)	AIDS, Kaposi's sarcoma, ARC	IND approved Phase II/III
Wellferon (alpha interferon)	Burroughs Wellcome (Research Triangle N.C.)	Kaposi's sarcoma, HIV, in combination with Retrovir	IND approved Phase I
Zovirax (acyclovir)	Burroughs Wellcome (Research Triangle Park, N.C.)	AIDS, ARC, in combination with Retrovir	IND approved Phase I

Immuno-Modulators

Drugs that enhance the body's ability to fight invaders

DRUG NAME (Generic Name)	MANUFACTURER	INDICATION	DEVELOPMENT STATUS
ABPP (bropirimine)	Upjohn (Kalamazoo, Mich.)	Advanced AIDS, Kaposi's sarcoma	IND approved Phase II/III
AS-101	Scientific Testing (National Patent Development, Bar Ilan University, Israel) (New York, N.Y.)	AIDS	IND approved
Ampligen (mismatched RNA)	DuPont (Wilmington, Del.), HEM Research (Rockville, Md.)	ARC, PGL	IND approved Phase III
(anti-human alpha interferon antibody)	Advanced Biotherapy Concepts (Rockville, Md.)	AIDS, ARC	IND approved Phase I
Carrisyn (acemannan)	Carrington Labs (Irving, Tex.)	ARC	IND submitted
Colony Stimulating Factor (GM-CSF)	Sandoz (East Hanover, N.J.) Genetics Institute (Cambridge, Mass.)	AIDS, Kaposi's sarcoma, ARC, HIV	IND approved Phase I
CL246,738	American Cyanamid (Pearl River, N.Y.)	AIDS	IND approved Phase I/II
(gamma interferon)	Genentech (South San Francisco, Calif.)	ARC, in combination with TNF (tumor necrosis factor)	IND approved clinical trials
IMREG-1	Imreg (New Orleans, La.)	AIDS, Kaposi's sarcoma, ARC, PGL	IND approved Phase III
IMREG-2	Imreg (New Orleans, La.)	AIDS, Kaposi's sarcoma, ARC, PGL	IND approved Phase II
Imuthiol (diethyl dithio carbamate)	Merieux Institute (Miami, Fla.)	AIDS, ARC	IND approved Phase II/III
IL-2 (interleukin-2)	Cetus (Emeryville, Calif.)	AIDS, Kaposi's sarcoma	IND approved Phase II
IL-2 (interleukin-2)	Hoffmann-La Roche (Nutley, N.J.), Immunex (Seattle, Wash.)	Kaposi's sarcoma	IND approved Phase III
INTRON-A (interferon alpha)	Schering-Plough (Madison, N.J.)	Kaposi's sarcoma	NDA filed
Isoprinosine (inosine pranobex)	Newport Pharmaceuticals (Newport Beach, Calif.)	ARC, PGL, HIV seropositive asymptomatic patients	IND approved Phase III
(methionine-enkephalin)	TNI Pharmaceuticals (Chicago, Ill.)	AIDS, ARC	Investigator's IND approved Phase I/II
MTP-PE (muramyl-tripeptide)	Ciba-Geigy (Summit, N.J.)	Kaposi's sarcoma	IND approved Phase I
Thymopetin (TP-5) (thymic compound)	Ortho Pharmaceuticals (Raritan, N.J.)	HIV infection	IND approved Phase I/II
Roferon-A (interferon alpha)	Hoffmann-La Roche (Nutley, N.J.)	Kaposi's sarcoma	NDA filed
(recombinant erythropoietin)	Ortho Pharmaceuticals (Raritan, NJ)	severe anemia associated with AIDS and AZT therapy	IND approved Phase II
Trexan (naltrexone)	DuPont (Wilmington, Del.)	AIDS, ARC	early Phase II
TNF (tumor necrosis factor)	Genentech (South San Francisco, Calif.)	ARC, in combination with gamma interferon	IND approved clinical trials

Anti-Infectives

Drugs that fight opportunistic infections

DRUG NAME (Generic name)	MANUFACTURER	INDICATION	DEVELOPMENT STATUS
Pentam 300 (pentamidine isethionate, IV dosage)	LyphoMed (Rosemont, Ill.)	PCP	NDA approved
aerosol dosage		PCP prophylaxis, PCP treatment	IND approved Phase III
(fluconazole)	Pfizer (New York, N.Y.)	cryptococcal meningitis, candidiasis	IND approved Phase III

SOURCE: Pharmaceutical Manufacturers Association

HOW DRUGS ARE APPROVED

Approval of a new drug for sale in the United States usually takes years.

Once a promising new chemical compound is found biologically active and safe in laboratory and animal tests, the drug company applies to the Food and Drug Administration for permission to begin clinical testing—tests on humans. The application, called an **investigational new drug** or **IND** petition, becomes effective if FDA does not disapprove it within 30 days.

Clinical testing has three phases:

Phase I studies, conducted on a small number of healthy people, determine the drug's pharmacological effect, its safe dose and how it is absorbed, metabolized and excreted by the body. Phase I usually takes less than a year.

Phase II tests are controlled studies in some 200 to 300 volunteer patients to assess the drug's effectiveness. They usually take about two years.

Phase III consists of clinical trials in large numbers of volunteer patients, usually 1,000 to 3,000, who have the condition the drug is intended to treat. The studies confirm the drug's effectiveness and identify adverse reactions. Phase III usually lasts about three years.

After Phase III trials, the drug company sends the FDA a **new drug application** or **NDA**, including all the data gathered in laboratory, animal and human studies, as well as production details and proposed labeling. NDA review and approval by FDA takes an average of two to three years.

Once the NDA is approved, the company can begin marketing the drug. It must submit periodic reports to the FDA, including data on adverse reactions, production and quality control. For some drugs, FDA requires additional monitoring and studies to evaluate long-term effects.

Other terms in the chart:

AIDS—acquired immune deficiency syndrome. **ARC**—AIDS-related complex, symptoms that often precede AIDS. **CMV**—Cytomegalovirus, an opportunistic infection that can cause blindness. **Orphan drugs**—Drugs for rare diseases; a new law provides federal subsidies for development of some such drugs. **PCP**—Pneumocystis carinii pneumonia, an often fatal opportunistic infection. **PGL**—persistent generalized lymphadenopathy, or lymph node enlargement.

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To: Jim Liverman / Jack Kasnowski

From: John Fraser

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CHEMICAL & ENGINEERING NEWS

A C&EN SPECIAL ISSUE

AIDS The science of a human tragedy

John Fraze

The Problem of Diagnostic Tests

In a perfect world, a person infected with human immunodeficiency virus (HIV) would always show up positive in a blood test; an uninfected person would always show up negative. What could be simpler?

Unfortunately, life is not simple. And present-day tests to detect HIV infection are far from perfect. Some individuals who aren't infected show up positive. Such a result, called a false positive, can lead to needless anxiety and depression, problems with interpersonal relations, discrimination, loss of home and job, or even suicide. That's because persons who harbor the virus are likely to develop—and die of—AIDS. On the other side of the fence are those infected individuals who falsely test negative. They breathe a sigh of relief and may proceed, unknowingly, to infect others, either by donating blood, engaging in unprotected sex, or sharing a needle to inject street drugs. Simply put, false positive and false negative test results destroy lives.

With wider HIV testing looming in the future, medical researchers are striving to eliminate such misleading test results by making HIV diagnostic tests more sensitive and more specific. Sensitivity is the ability to detect low levels of a target molecule; specificity is the ability to detect the target molecule exclusively. In addition, researchers are developing new tests that will be quicker, simpler to perform, and less expensive than those currently available.

The tests now in use or under development are of two basic types. The type that came into use first is an indirect test: It detects antibodies (proteins called immunoglobulins) that the immune system has mobilized against the invading AIDS virus. The second type of HIV test is one that detects the virus directly, either through its antigens or its genetic material.

Deficiences of current HIV tests are being addressed by development of new kinds of tests for antibodies, antigens, and nucleic acids

Ron Dagani, C&EN Washington

The most widely used antibody test is the enzyme-linked immunosorbent assay (ELISA). This test can screen large numbers of blood samples. It was rushed into the marketplace to protect the blood supply, which had already been infiltrated by the AIDS virus. ELISA was purposely designed to be oversensitive—to catch even "suspicious" blood samples. As a result, it produces many false positives. In fact, according to a study by Harvard University researchers, using ELISA to screen persons at low risk of infection "yields many more false positives than true positives."

All blood samples that test positive are retested using ELISA. Those that test positive again are subjected to a different, more definitive test called the Western blot. Unlike ELISA, which is a yes-or-no test, the Western blot actually reveals the antibody profile of a blood sample. Thus, it is considered a more accurate indicator of the presence of HIV antibodies. If the Western blot is positive, the person is considered to be infected.

The Western blot produces fewer false positives than ELISA, but it's not infallible. Scientists estimate that, at the end of this testing process, one to three individuals out of 100,000 in a low-risk population will be incorrectly told that they are infected with HIV. The false positive rate is lower in groups that have a high prevalence of HIV infection, such as urban homosexual men and drug addicts.

Other assays do exist to confirm a positive ELISA, but these aren't practical for large-scale use. The radioimmunoprecipitation method, for example, is sensitive and specific, but it involves radioactivity and requires considerable technical skill. Virus culturing is the most specific method, but it is difficult, time-consuming, and also requires highly skilled personnel.

Blood banks and other HIV testing centers in the U.S. currently can choose from seven ELISA test kits that have been approved for commercial use by the Food & Drug

Technician uses Du Pont HIV antigen test to monitor viral activity

Administration. These kits are available from Abbott Laboratories, Cellular Products, Du Pont, Electro-Nucleonics, Genetic Systems, Organon Teknika, and Ortho Diagnostic Systems. Du Pont is unique in that it is the only U.S. firm whose Western blot test also has been licensed for clinical use. Du Pont's ELISA and Western blot tests were both developed in partnership with Biotech Research Laboratories of Rockville, Md. Western blot kits from other firms are being used in research and may be approved by FDA for wider use.

Assays like these detect HIV antibodies by capturing them on antigen-coated surfaces and using a color reaction to visualize the antigen-antibody complex. The commercially available ELISAs, which are called first-generation tests, use disrupted whole-virus preparations as the antigen. These preparations, though made from purified virus, are contaminated with cellular debris that can attract non-HIV antibodies and thus produce false positives.

Second-generation tests

Scientists now are developing second-generation tests that use one or more genetically engineered HIV antigens instead. Because these preparations are purer, the number of false positives is reduced. Another benefit is that the test manufacturer avoids the hazard of working with live virus.

Some of these second-generation tests are said to be so reliable and easy to use that they may allow rapid HIV screening to expand into new markets, such as developing nations, physicians' offices, and possibly even the home market. For instance, at the University of California, Davis, a team of medical researchers has developed a dot enzyme immunoassay (dot EIA) on a small plastic card. The assay detects antibody to gp41, the glycoprotein that spans the outer membrane of HIV. In this assay, a drop of serum is exposed to recombinant gp41 on the card. If anti-gp41 antibody is present, it binds to the antigen. After detection reagents are applied, the bound antibody signals its presence by the appearance of a blue dot.

According to James R. Carlson, an assistant professor of pathology and internal medicine who leads the UC Davis team, the prototype of this dot EIA was more than 99% accurate in preliminary evaluations using blood sera from foreign and U.S. sources. These results compare favorably with ELISA and Western blot results, he says. Moreover, the assay takes about 30 minutes to perform, compared with two to four hours for the typical ELISA. It's also less expensive than the ELISA—about 25 cents versus $1.00 to $4.00 for ELISA kits.

A test like the dot EIA seems to be tailor-made for remote areas that lack highly skilled technicians or laboratory facilities, such as Africa. In some African cities, as many as 18% of the blood donors have been found to be HIV-infected, Carlson says, "yet donor blood isn't usually screened due to technical and economic constraints." The test is being developed for clinical use in a joint venture between Virotechnology Laboratories of Stockton, Calif., and Bio-Medican Corp. of Huntington Beach, Calif.

An even faster second-generation test has been produced by Cambridge BioScience, a biotechnology firm based in Worcester, Mass. The basis of this test is Recombigen, a novel recombinant antigen that attracts antibodies to both gp41 and gp120, the virus's envelope glycoprotein. "All of our data show that envelope antibody is the earliest and most important marker for detecting HIV antibody," says Rod N. Raynovich, vice president for business development at Cambridge BioScience.

The firm's test involves mixing a drop of blood serum with a drop of latex reagent on a plastic card. The reagent contains microscopic latex beads coated with Recombigen. If antibodies to the envelope proteins are present in the sample, the latex beads clump. The resulting clusters become visible in minutes as small white dots. If clumping has not occurred within five minutes, the sample is considered HIV-free.

In June, Thomas C. Quinn of the immunoregulation lab of the National Institute of Allergy & Infectious Diseases (NIAID) reported at the 3rd International Conference on AIDS in Washington, D.C., that the Recombigen latex agglutination assay had been tested on sera from 2000 patients residing in Africa and the West Indies. Those tests indicated that the assay was accurate more than 99% of the time, and was more specific than a single ELISA. Quinn also said that the latex test was faster and simpler than the ELISA, and reliable enough that confirmatory tests might not be needed. Raynovich believes the Recombigen assay is better than the Western blot, and could replace it as a backup test.

Cambridge BioScience hopes to receive government approval to begin marketing the latex test in 1988 for use in physicians' offices and other low-volume, "decentralized" testing sites. The firm began conducting clinical trials of the test in September. Raynovich says the company plans to submit the results to FDA by the end of this year. In the meantime, FDA has allowed Cambridge BioScience to ship 50,000 test kits to Zaire, where the test is being used to screen for HIV-infected blood.

Murex Corp. of Norcross, Ga., also is developing a fast HIV screening test, but this one doesn't rely on a single recombinant protein. Rather, it uses seven natural HIV antigens that have been isolated by affinity purification using monoclonal antibodies, according to Gerald A. Bush, who has been leading the development effort. These antigens react with antibodies to the three major types of structural proteins in the virus—envelope, core, and replication-related proteins.

The reaction between these purified antigens and serum antibodies takes place inside a small, disposable, plastic cartridge. A positive reaction is indicated by the appearance, within 10 minutes, of a blue color in the cartridge's window.

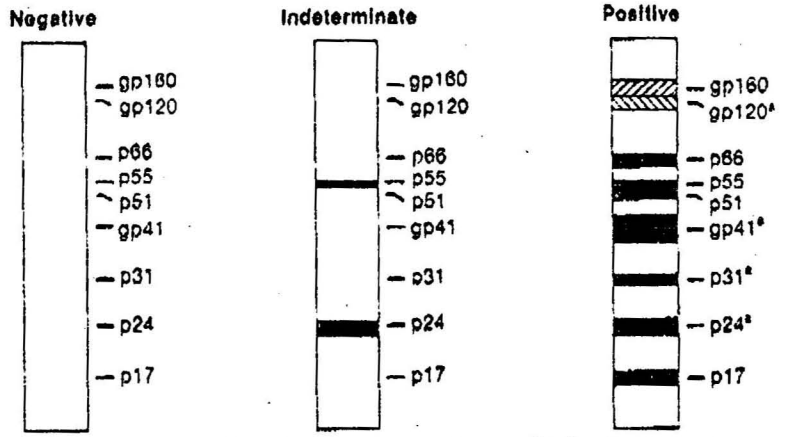
In early tests, the sensitivity and specificity of the Murex assay have been greater than 99%, says Bush. The assay actually picks up seropositive blood earlier than the ELISA, he notes. That might be because the purified antigens are so highly concentrated that they can detect lower levels of antibody, he explains. Bush says Murex expects

How ELISA and Western blot tests work

The screening of blood samples for antibodies to HIV (the AIDS virus) usually starts with an enzyme-linked immunosorbent assay, or ELISA. In this assay, blood serum is diluted and placed in microtiter wells or on beads that have been coated with HIV antigen (derived from disrupted virus that has been grown in cell culture). If the specimen contains antibodies (immunoglobulins), they will bind to the test kit's antigens. After an incubation period of about two hours, any unbound antibody is washed away. An enzyme-labeled antibody to human immunoglobulin is added, and this binds to any HIV antibodies that have become bound to antigen. The bead or well is again washed to remove any unbound antibody. A substrate is added and the enzyme bound to the antihuman antibody catalyzes a reaction that produces a color change. The optical density is measured spectrophotometrically and is directly related to the amount of HIV antibodies in the sample. The absorbance reading is interpreted by comparing it to positive and negative control samples containing known quantities of antibodies.

Samples that repeatedly test positive by ELISA are subjected to a different, more expensive procedure called the Western blot. This test identifies antibodies to the major HIV antigens. These antigens, derived from purified disrupted virus, are fractionated by size using polyacrylamide gel electrophoresis by the test's manufacturer. The fractionated viral proteins are then transferred onto nitrocellulose paper. A serum sample is applied to a strip of the paper. If antibodies to HIV are present, they will bind to the viral antigens. An enzyme-labeled antibody to human immunoglobulin is applied to the paper to bind to any HIV antibodies bound to antigen. An appropriate substrate is added and the enzyme catalyzes the color reaction. If HIV antibodies are present, a pattern of distinctive bands appears on the strip. The location of each band indicates reaction to a specific viral protein and is compared visually with a Western blot from a specimen in which antibodies to all of the HIV proteins are present. A positive result on this test almost always means the blood sample contains HIV antibodies.

Western blot readings have three possible interpretations



Source: Du Pont

to begin clinical trials of the assay by the first of the year, and the test to be licensed in 1988.

Home test for HIV?

A special feature of Carlson's dot EIA and the Cambridge BioScience and Murex rapid tests is that they work with whole blood as well as serum, according to sources involved in their development. That means they have the potential to be over-the-counter or home tests. But scientists and company officials discount this possibility, at least for now. They worry about the medical and ethical implications of AIDS testing in the home. Positive test results can devastate people, and any test could give a false positive, they say. "It's like giving somebody a death sentence," remarks one company spokesman.

Health care professionals agree that people need proper counseling to correctly interpret the results, plan confirmatory tests, and obtain medical and psychological therapy, if needed. "We believe a physician must be in the loop," says Raynovich. "We don't think FDA would approve a home test [for HIV] in the near term."

Similar thinking prevails at Du Pont, which is clinically evaluating an HIV antibody test the size of a matchbook. This test, like its potential competition, uses a color change to give the same yes-or-no information as ELISA, only much quicker (in five minutes). Du Pont spokesman Mike Ricciuto says the firm hopes to begin marketing the matchbook test in Africa later this year. In the future, he says, Du Pont might seek FDA approval to sell the test to hospitals and physicians in the U.S. "It would probably never be marketed as a home test," he adds.

Because any of these screening tests could produce false positives, most scientists believe that there's still a need for confirmatory assays such as the Western blot. This test, sometimes called the immunoblot, provides more information than the standard ELISA because it exposes a serum sample to a strip of paper containing all the key HIV antigens separated according to their molecular weight. Thus, the identity and relative abundance of specific antibodies in the sample can be

visualized on the strip according to the specific antibody-antigen reactions that occur.

Problems with Western blot

Nevertheless, indeterminate and false positive results can arise on the Western blot because the method is not standardized. A 1986 Institute of Medicine report on AIDS noted that "There has been considerable variation in what different laboratories have interpreted as positive in Western blots." In the past, some testing labs have considered antibody to the core protein p24 as sufficient for a positive result. The Institute of Medicine report states that this is an equivocal result. The National Institutes of Health requires antibody to p24 and to the transmembrane protein gp41. Du Pont says a positive result must include p24, p31 (a replication-related protein), and either gp41 or gp120.

Improvements in the Western blot have helped to some extent. Earlier versions of this assay were deficient in detecting antibodies to gp120 because this key protein was selectively lost during the manufacturing process. The newer versions of the test, such as Du Pont's, offer enhanced detection of antibodies to gp120 and its precursor protein gp160, making it easier to classify "some potentially ambiguous sera," says Stanley H. Weiss of the National Cancer Institute's environmental epidemiology branch.

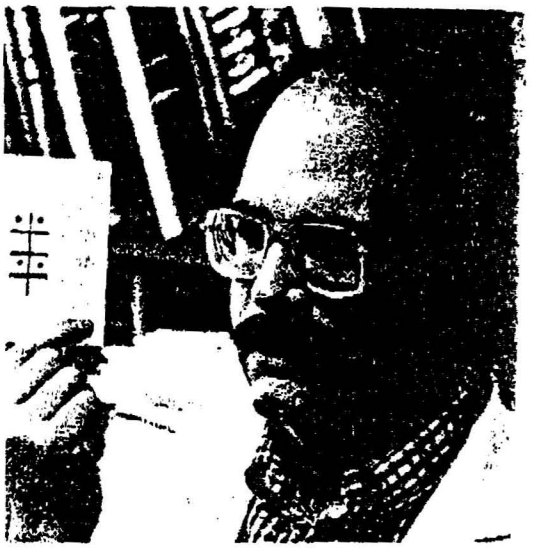
Even so, the interpretation of Western blot reactions can still be problematic. Weiss notes, for example, that some persons who had "unusual Western blot reactivity patterns" or showed no evidence of HIV antibodies were found to be HIV-infected by virus culture. As a precaution, blood banks currently reject donations that repeatedly test positive by ELISA, even if they test negative in the Western blot. Many of these ELISA results certainly are false positives, and the blood that is thrown out is wasted.

In an effort to clear up the uncertainty in some Western blot readings, scientists at Abbott Labs have devised an immunoassay that is complementary to the Western blot. This assay, Envacor, actually consists of two tests: One detects antibody to an envelope protein (gp41) and the other detects antibody to a core protein (p24).

Envacor may be useful as a diagnostic tool because the two antibody types provide different information. "Envelope antibodies are present in virtually everybody who's infected," says Jean-Pierre Allain, who manages Abbott's research on AIDS diagnostics. They are "an extremely stable and reliable marker for HIV infection" throughout the course of the disease, he adds. Antibodies against p24, by contrast, are not always present and their level changes depending on the stage of infection. About 85 to 90% of infected asymptomatic persons have anti-p24 antibodies. But as these individuals become sicker, anti-p24 levels drop lower and lower, eventually becoming undetectable. Thus, Envacor might be useful in charting and predicting the immunological course of the disease, says Carlson, who directs the AIDS Virus Diagnostic Laboratory at UC Davis.

The Abbott test offers a number of advantages over the Western blot, according to Allain. Envacor is easier to perform because it's in an ELISA format. It gives a numeric readout of results, thus avoiding the subjective interpretations required by Western blot. It's also quicker, although it requires an overnight incubation step like the Western blot.

Allain: immune system collapse



University of California's Carlson holds the dot EIA test for HIV

On average, the two tests are of similar sensitivity. But Envacor's specificity is very high—99.9%. This means that, statistically, 999 out of 1000 HIV-negative blood samples will be unreactive in the test. Only one in 1000 is likely to turn up as a false positive. In Europe, where Envacor has been in use for more than a year, Abbott recommends that it be used in conjunction with the Western blot.

Tests for viral antigen

The variability of the immune system's antibody response to HIV proteins in different people has led researchers to develop a third generation of AIDS tests, which detect the virus or its fragments directly. This approach promises to catch some of the false negative results that crop up in antibody tests.

False negatives occur in persons who haven't yet developed detectable levels of HIV antibodies. Typically, antibodies aren't detectable in blood until six to 10 weeks after infection, according to Jaap Goudsmit, head of the retrovirus laboratory at the University of Amsterdam. A recent study of homosexual men in Finland has found that some HIV-infected persons don't develop antibodies for a year or more. And some HIV carriers may never mount an antibody response at all. Even so, these individuals are still considered infectious, and their blood may contain detectable levels of antigens.

Obviously, one way to pick up infected blood earlier than is possible with antibody tests would be to look for viral antigens. Several companies are developing such antigen tests for marketing. Abbott and Du Pont unveiled their entries in June at the 3rd International Conference on AIDS. Although their antigen tests aren't yet available commercially in the U.S., they have been used as research tools for at least a year.

The Abbott and Du Pont antigen tests are both enzyme immunoassays that directly detect the core protein p24, which is shed by the virus. The Abbott test, for instance, uses purified, naturally occurring antibodies from an infected individual. These are bound to a solid phase and are allowed to capture whatever antigen may be in the sample. The visualization chemistry is similar to that used in other enzyme immunoassays.

Researchers stress that such direct-virus tests will complement, not replace, antibody tests. That's because the levels of antigens and antibodies change with time, and looking at only one or the other can give a misleading result. For example, if a person is producing antibody, then antigen in the blood will be masked to some extent by the binding of the two, Du Pont's Ricciuto explains. In this instance, antigen may not be detected at all, although an antibody assay would correctly signal HIV infection. Hence, it would be necessary to screen blood for both antibody and antigen, he says.

The rationale for adding the antigen test to blood screening programs is to eliminate donations of infected but antibody-negative blood. However, no one really knows whether this test will be able to pick up infected blood early enough to warrant the additional cost, says Ricciuto. According to John P. Phair, professor of medicine at Northwestern University Medical School in Chicago, since blood screening for HIV was instituted in 1985, scientists have documented one case in which HIV was transmitted by transfusing antibody-negative blood. Phair noted in a recent editorial in the *Journal of the American Medical Association* that blood banks would have to spend at least $5.00 to $7.00 per unit of blood to test for antigen. The added expense is not justified at this time, he concluded.

The antigen test might be more important as a way to diagnose AIDS earlier than is now possible. And

Diagnostic tests being developed for other retroviruses

in the U.S., as far as anyone knows, all cases of AIDS have been caused by the same retrovirus—a human immunodeficiency virus (HIV-1).

In Africa, the situation is more complicated. HIV-1 is widespread in Zaire and other central African nations. But a closely related human retrovirus, HIV-2, is endemic in Senegal and several other countries in Western Africa. HIV-2 appears to cause an AIDS-like disease. Both HIV-1 and HIV-2 are highly prevalent in at least two West African countries. Furthermore, Africa also is the home of simian immunodeficiency virus (SIV), which appears to be more closely related to HIV-2 than to HIV-1. SIV doesn't infect humans, but it does cause an AIDS-like disease in some monkeys.

The prevalence of infections caused by these viruses in Africa appears "catastrophic," according to a research team that includes Erling Norrby of the Karolinska Institute in Stockholm and Richard Lerner of the Research Institute of Scripps Clinic in La Jolla, Calif. They say there is "an emergency need" for simple, inexpensive, sensitive, and specific tests that can identify antibodies to these retroviruses, and distinguish between them.

Several groups have been working to develop such immunoassays, and at least two—Norrby's and a different group headed by John W. Gnann Jr. of Scripps Clinic—recently reported success. Both groups have based their assays on synthetic peptide antigens derived from the transmembrane glycoproteins of the different viruses [*Nature*, **329**, 248 (1987); *Science*, **237**, 1346 (1987)]. These antigens were incorporated into the standard laboratory screening format known as ELISA. Besides pointing the way toward better diagnosis of AIDS infections, early results from this research offer hope that it will be possible to solve what Gnann's team calls "the epidemiologic puzzle of AIDS in Africa."

Researchers also have been testing blood from American donors to check if HIV-2 has spread to the U.S. So far, no evidence of this has turned up. For now, it seems, HIV-2 tests will remain for investigational use only.

Scientists and health officials are more concerned that another insidious retrovirus may be spreading through the U.S. blood supply—HTLV-1, or human T-cell lymphotropic virus, type 1. This virus causes adult T-cell leukemia in about 1% of those infected. Like AIDS, this cancer is fatal and may not appear until years after infection. HTLV-1 is transmitted in the same way as HIV—through sexual contact, exchange of blood by hypodermic syringes, and transfusion of infected blood or blood products. Evidence indicates that many drug addicts in the U.S. have already been infected with HTLV-1, which is also implicated in other diseases.

Cellular Products, a Buffalo, N.Y., biotechnology company, and Du Pont, in collaboration with Biotech Research Laboratories of Rockville, Md., have produced experimental test kits to detect HTLV-1. The American Red Cross has been using these tests to look for evidence of the virus in blood donated by people around the U.S. The results of this study aren't yet available.

Nevertheless, many companies already see the writing on the wall. At present, all blood donations in the U.S. are screened for HIV antibodies and "we believe it is only a matter of time" before blood screening for HTLV-1 also will be required, says John R. Zeman, vice president and general manager of Eastman Kodak's clinical products division. Kodak plans to market a test manufactured by Cellular Products that can simultaneously detect HIV and HTLV-1 antibodies. Du Pont, too, is already gearing up to produce millions of HTLV-1 antibody test kits a year.

because p24 and anti-p24 levels in blood change as the disease progresses, the antigen test, like Abbott's Envacor assay, may be useful in predicting the course of the disease. Goudsmit says detectable p24 can appear as early as two weeks after infection. Weeks later—estimates range from six to 12 weeks after infection—core antigen disappears, perhaps overwhelmed by a rising tide of antibodies against it. But in some persons who go on to develop full-blown AIDS, researchers say, p24 reappears later, signaling the renewed activity of the virus. Because antigen complexes with antibody, a rising level of p24, coupled with a falling level of anti-p24, "reflects the failure of the immune defenses to contain the virus," Allain says.

A recently published study by researchers at Abbott Labs and Rush-Presbyterian—St. Luke's Medical Center in Chicago indicates that HIV p24 levels correlate significantly with the clinical status of persons infected with HIV. The study looked at 221 infected persons, 130 of whom tested positive for HIV antigen. Antigen was detected in 19% of the asymptomatic subjects, 46% of those with AIDS-related complex (a less severe form of the disease), and 69% of the AIDS patients. A related study also used the Abbott test to find the first indication of HIV infection in six subjects who had no detectable HIV antibodies. These and other recent studies suggest that the antigen test could detect HIV infection at an earlier stage and could help identify which infected people are most likely to develop full-blown AIDS.

Certainly, the antigen test is expected to be an essential tool for monitoring the efficacy of experimental anti-HIV drugs by measuring antigen levels after drug administration. According to Paul A. Volberding, who directs AIDS activities at San Francisco General Hospital, the Abbott antigen test helped researchers determine that the drug zidovudine (popularly known as AZT) retards the virus's replication in the body. This was seen in declining levels of p24, which is used as a marker of viral burden.

In some HIV-infected persons, the virus retreats into the cells and lies dormant for months or years until it is reactivated. During this latency period, the virus cannot be cultured from the blood, and neither viral antigens nor antibodies are detectable. The only way to unmask the virus is to find the telltale genetic sequence it has surreptitiously inserted into the DNA of host cells.

Researchers are applying nucleic acid probes to accomplish this mission. Such a probe consists of a synthetic sequence of nucleotides—either RNA or DNA—that is complementary to the target viral sequence. When the probe encounters its target, the two strands hybridize, or join together to form a double-stranded segment. Because the probe sequence is tagged with a radioactive or nonradioactive label, the site of the target sequence can be located.

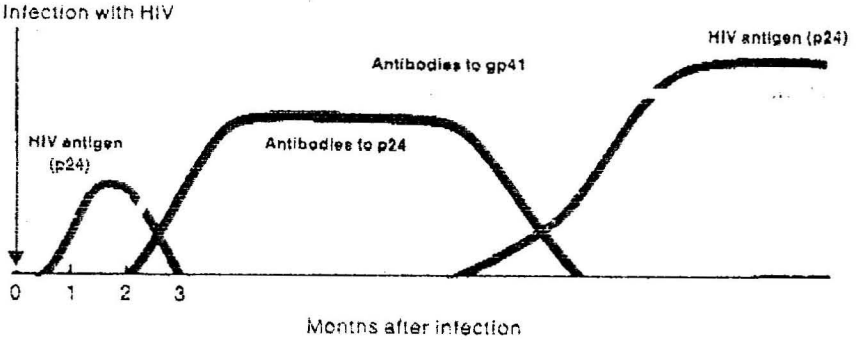
Nucleic acid probes theoretically could detect HIV infection even earlier than antigen tests. Practicably, though, many problems exist. For example, it's estimated that only one in 10,000 cells typically is infected and produces viral RNA protein. Moreover, infected cells carry only a limited number of copies of the viral sequence (usually one to three). At very early stages of infection especially, a probe would have to be extremely sensitive to pick up such small amounts of viral DNA.

Researchers at Cetus Corp., a biotechnology firm in Emeryville, Calif., have gotten around this problem by developing a method to amplify the viral DNA sequence a million times (C&EN, April 21, 1986, page 8). John J. Sninsky, director of diagnostics at Cetus, explained it this way to a newspaper reporter: "If looking for the AIDS virus is [like] looking for a needle in a haystack, our procedure allows you to make a million needles." Cetus claims the procedure can detect viral genes if present in only one of every 5000 cells.

Even so, the use of such probes requires a more sophisticated lab setup than do most other HIV tests. This is limiting the use of these probes. Furthermore, some scientists believe that the promise of this tool will not arrive until "somebody figures out a way to automate it," says Du Pont's Ricciuto.

Although researchers now can test for antibodies, antigens, and even bits of viral genes, they still don't have a clear idea of what the test results mean in terms of the patient's clinical condition and prognosis. Perhaps, one diagnostics analyst tells C&EN, that all-important connection will be made once scientists have in place a battery of assays that can be run routinely on AIDS patients. □

Core antigen, antibody levels vary as disease progresses



A hypothetical patient's profile shows the inverse relationship between two key HIV markers—the core antigen p24 and antibodies raised against it. p24, which appears in blood soon after infection, is neutralized by a rising tide of anti-p24 antibodies. As long as the level of these antibodies remains high, the patient's condition is stable. But months or years later, when the virus starts replicating again, its stepped-up production of p24 overwhelms the immune defenses by complexing all the available anti-p24 antibodies. By contrast, the level of antibody to the transmembrane glycoprotein gp41 remains fairly constant.

Source: Abbott Laboratories

New York Times - 2/14/88

Spread of AIDS Abating, But Deaths Will Still Soar

By PHILIP M. BOFFEY
Special to The New York Times

WASHINGTON, Feb. 13 — As the AIDS epidemic moves into its eighth year in the United States, the evidence grows ever stronger that the much-feared explosive invasion of the general population is not occurring, and never will.

Still, there is nothing on the horizon that can avert a dramatic rise in disease and death over the next few years, the inevitable result of the wildfire spread of the AIDS virus in the early 1980's.

A wide range of data amassed over the past year reveal that the virus has stopped spreading in surveyed groups of gay men. In several cities it continues to infect black and Hispanic drug addicts, their sex partners and their babies at a disturbing rate.

A Shift in Views

But "we do not expect any explosion into the heterosexual population," Dr. Otis R. Bowen, Secretary of Health and Human Services, said last month, in a striking shift in views. Only a year ago he warned that the disease was "rapidly spreading" to the wider population and would ultimately make the Black Plague, which wiped out a third or more of Western Europe's people in the 14th century, seem "pale by comparison."

The change in attitudes at the very top of the Federal health establishment reflects the accumulating message of hundreds of studies, large and small, that have now been completed.

Despite the new evidence that the spread of the AIDS virus may be moderating, the nation must still live through an awful, escalating toll of fatal cases of acquired immune deficiency syndrome.

Cities like New York and San Francisco are already mobilizing to cope with a sharp rise in AIDS cases in the next few years that will dwarf anything seen thus far.

In San Francisco, a huge part of the male homosexual population will be wiped out, altering the demographics and politics of a city that has become a haven to gay men. In New York, so many drug addicts, their sexual partners and their babies will become ill that health care costs for them and for gay victims of AIDS will soar above $1 billion a year in 1991. Scarce hospital beds will be crammed or overflowing.

This upsurge in illness is unavoidable even if the virus should completely stop spreading. It often takes seven years or more after infection with the virus before symptoms of disease develop; most of the million or more Americans who were infected in the early 1980's have not yet be-

Continued on Page 36, Column 1

AIDS: Confronting The Epidemic

"The way mankind responds to crisis is first disbelief, then denial, then the third stage is mobilization, and we're at the horizon of that now."

Dr. Stephen C. Joseph
New York City Health Commissioner

First in a series of four articles.

Spread of AIDS Virus Has Slowed, but Deaths Will Still Soar

Continued From Page 1

come ill, although, evidence indicates, a majority of them will. Available drugs will prolong the lives of some but not all victims.

'Of Biblical Proportions'

Government projections suggest that as many as one in every 30 American men 20 to 50 years old, most of them gay, are already infected with the virus. Unless a cure is quickly found, recent evidence indicates, a majority of them will develop AIDS and eventually die.

"This, is a huge epidemic by anybody's standards," said Dr. George Rutherford, medical director of the AIDS Office of the San Francisco Department of Health. "It would not be incorrect to describe it as of biblical proportions."

Dr. June Osborn, dean of the school of public health at the University of Michigan, said, "The trends are awful." Citing projections that the caseload will soar by the end of 1991, She continued: "We have only three and a half years to be bracing for a real devastating epidemic in young adults. In the 25- to 45-year age group, everybody is going to know somebody suffering from AIDS."

Portrait Of the Crisis

Although there are still many gaps in knowledge, Government health officials have amassed enough data to give them a reasonably clear picture of the crisis.

Their new portrait of the epidemic, presented in a report to the White House from the Federal Centers for Disease Control at the end of last year, is built from hundreds of studies from 1984 through 1987, including 50 studies of gay and bisexual men, 88 studies of intravenous drug users, 33 studies of hemophiliacs, 21 studies of the heterosexual partners of infected individuals, 27 studies of women and scattered studies of prostitutes and prisoners.

Many of these studies were relatively small, but their findings were buttressed by large-scale screening of more than 25 million blood and plasma donations, more than 1.2 million applicants for military service, more than 2 million active-duty members of the military services, and tens of thousands of Job Corps applicants and newborn infants.

The Federal Government is initiating new surveys to look for the virus in 30 major metropolitan areas and is exploring the possibility of a nationwide sampling of the entire population.

The New York Times/Terrence McCarthy

Dr. George Rutherford
Medical director of the AIDS Office of the San Francisco Department of Health.

"This is a huge epidemic by anybody's standards. It would not be incorrect to describe it as of biblical proportions."

"I don't think there are any huge shocks in store," said Dr. Andrew Moss, an epidemiologist at San Francisco General Hospital. "I don't think there will be any awful surprises as we get more information."

The Sureness Of Devastation

As of Feb. 1, about 52,000 cases of AIDS had been reported in the United States. But more cases than that are expected to be identified in the single year of 1991, according to Government projections.

These projections, first put together in June 1986, have thus far proved on target, giving Government experts great confidence that they know where the epidemic is headed in the short run.

In making the projections, the extrapolated from previous trends to predict a cumulative total of 270,000 cases by the end of 1991. They also noted that the real toll might be at least 20 percent higher because of failure to report or diagnose many cases.

Thus far the actual cases have run just slightly below the projections. "We've had about 92 percent of the number of cases that we projected would be reported by this time," said Dr. W. Meade Morgan, chief of AIDS statistics at the Centers for Disease Control in Atlanta. "It's encouraging that the number is not higher than we thought. But part of the shortfall, we fear, is that case reporting may not be as good as it once was."

Staggering Toll Likely

A broadening of the definition of AIDS last year, to include neurological symptoms, emaciation and certain infections, could drive the volume of AIDS cases reported by 1991 above the projections made in 1986.

The toll of sickness and death in the next several years is apt to be staggering in the most heavily afflicted communities.

In San Francisco, Dr. Rutherford said, "the epidemic is having a huge economic impact on the city, and it will have a huge demographic impact as well, because a large percentage of the population is going to die."

"Half the gay men in the city are infected and a majority of them will develop AIDS," Dr. Rutherford said. "That's a big chunk out of the population. It can't be minimized."

By some estimates, Dr. Rutherford said, 5 percent of the total population in San Francisco is infected, an infection rate equal to that of the most severely afflicted cities of Africa.

Top Cause of Death in Young

New York City is facing an equally daunting outlook, with no indication that any relief is in sight, according to Dr. Stephen C. Joseph, the city's Commissioner of Health. New York City has already found that far more deaths from AIDS are occurring among drug addicts than the official figures suggest, possibly two and a half times as many, making addicts the chief victims of AIDS in the city, with gay men slipping to second place.

AIDS is already the leading cause of death in New York among men 25 to 44 years old and women 25 to 34. More than 13,000 cases of AIDS have been reported in the city since 1981, but by the end of 1991, according to Dr. Joseph, the cumulative total may exceed 50,000 under the expanded definition of AIDS.

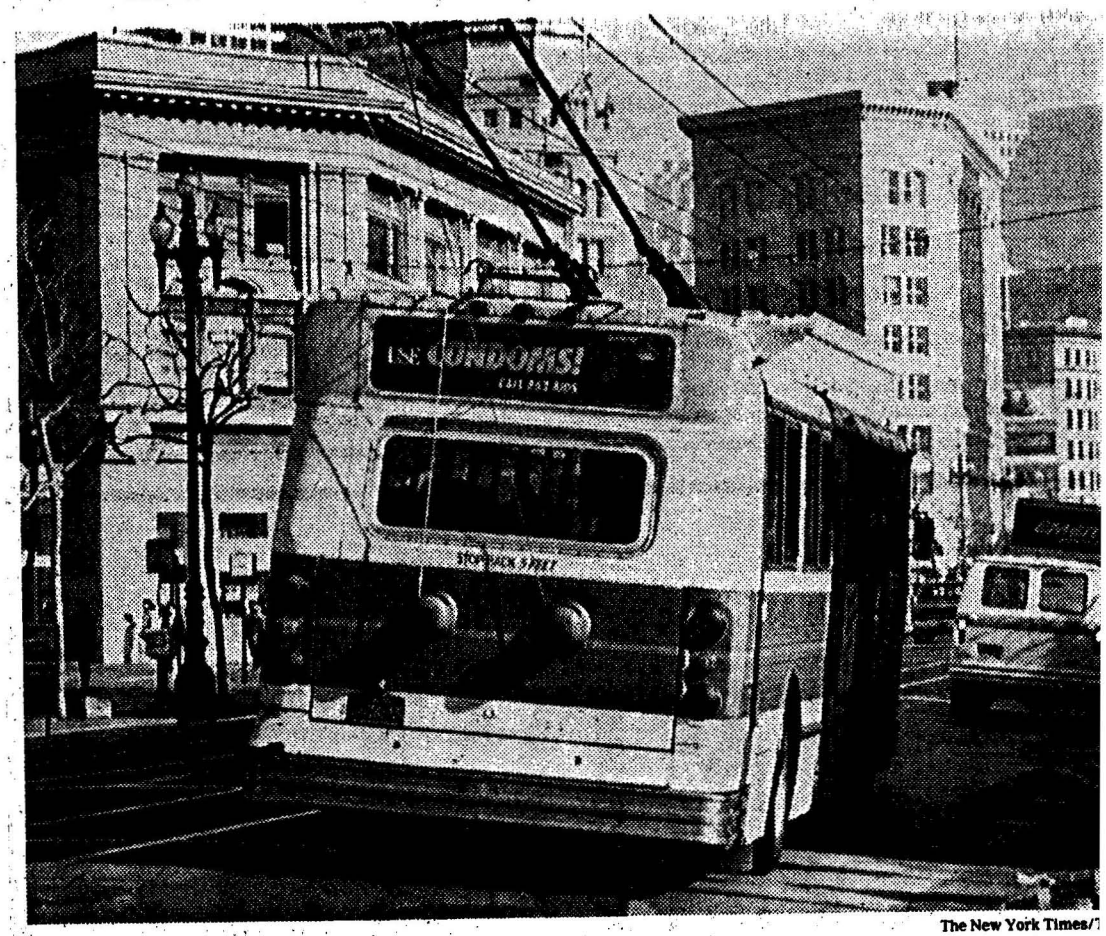
New York City will have enormous need for more hospital beds in both municipal and private institutions. The city now uses about 1,200 of its 25,000 public and private hospital beds for AIDS patients. But by 1991, Dr. Joseph said, they will will need more than 3,000 beds. Nursing beds will be even more scarce.

Tripling of Costs

The annual costs of treating people with AIDS in New York City will probably triple over the next three years, from about $385 million today to more than $1 billion annually by 1991. "The impact of AIDS on our citizens, our hospitals and our cities will be beyond that of any modern public health crisis," Dr. Joseph told a recent conference.

But nationwide, the impact will not appear large, according to an analysis of several cost studies by David E. Bloom, professor of economics at Columbia University, and Geoffrey Carliner, executive director of the National Bureau of Economic Research.

In the Feb. 5 issue of Science magazine, they said that the lifetime cost of medical care for the 270,000 cases projected for the decade 1981 to 1991 is not apt to exceed $22 billion. "By the early 1990's patients with AIDS will constitute a dramatically higher but nonetheless small fraction of total deaths in the United States," the article said. "Compared to total spending on medical care, or deaths from all illnesses, the national impact of AIDS in the early 1990's will be small."



The New York Times/T

A condom advertisement on the back of an electric bus in San Francisco.

Invasion Of the Virus

While the number of people sick with AIDS will continue to soar, the previous wildfire spread of the virus appears to have slowed and shifted its targets, changing the face of the epidemic. Whereas the epidemic in its early years primarily afflicted middle-class white gay men, it is now spreading more rapidly among poor blacks and Hispanics in the ghettoes of a few major cities, particularly among drug addicts and their sexual partners.

"The severity of the epidemic is catastrophic in the male homosexual population and also among intravenous drug users in the inner city," said Dr. Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases. "But what's encouraging is that we are not seeing any significant spread into the general population. It's likely that after 1991 we'll start to see a plateauing of cases and then a decrease, because the infection rate is already down."

Dr. David Axelrod, Commissioner of Health for New York State, told the President's AIDS Commission in December that the AIDS epidemic in New York appeared to have reached "a plateau with respect to the numbers of new cases being reported," at roughly 300 per month.

Feared invasion into the general population hasn't happened.

Estimate Essentially Flat

The Federal Government's official estimate of the number of Americans infected with the AIDS virus has remained essentially flat for the last year and a half. In June 1986, the Centers for Disease Control estimated that 1 million to 1.5 million Americans, the vast majority of them gay men or intravenous drug users, were infected.

In a report to the White House Nov. 30, 1987, the centers refined this estimate, to 945,000 to 1,400,000, a slight decrease even though the virus had presumably been spreading since the previous estimate. Projections by various mathematical models have ranged from a low of 276,000 Americans infected to a high of 1,750,000.

The AIDS epidemic, many experts say, can be understood only as a series of subepidemics, each progressing with its own dynamic.

In gay men, the virus spread rapidly before most people realized the danger, infecting half or more of sexually active gay men surveyed in cities like New York, San Francisco, Philadelphia and Denver.

But now there are signs that the rate of new infections in many gay groups has declined. In one group of 350 men in San Francisco, the rate of new infections per year peaked at 21 percent of the group in 1982 and fell to a single new infection in 1986 and in 1987.

"AIDS may kill half of the homosexual men in America's biggest cities," said Dr. Moss, the epidemiologist at San Francisco General Hospital. "But the epidemic in gay men is basically over in San Francisco."

In Drug Users, Fast Spread

In contrast, the epidemic spread of the virus in intravenous drug users continues at a high rate. In New York City, where new infections have recently been occurring in surveyed gay men at the rate of only 1 percent a year, they have struck drug addicts at a rate of 7 to 8 percent a year, according to state health officials.

The epidemic in drug users has remained highest in New York City, northern New Jersey and Puerto Rico, with an infection rate of 50 to 65 percent of various groups of drug addicts, and in cities with close geographic or cultural ties with these centers. Moderately high rates of infection have been found in small groups tested in such cities as Hartford, Conn., more than 40 percent; and Baltimore, 30 percent.But in most areas of the country, the rates among addicts are below 5 percent.

Once the virus becomes prevalent in a group of intravenous drug users, it appears to spread rapidly through the sharing of needles. But the infected drug addicts appear to be less mobile than many of the infected gay men and thus slower to spread the virus to distant cities.

For Most, Risk Is Slight

Heterosexual spread of the virus has thus far been confined largely to the sex partners of people who got the disease some other way: through needle sharing; through receiving infected blood products before the spring of 1985, when the blood supply was safeguarded, or through homosexual intercourse. Three small studies in New York and San Francisco found that 40 to 60 percent of the sex partners of infected addicts became infected, and 18 other studies of the heterosexual partners of a variety of other infected individuals found that 0 to 58 percent of the sex partners became infected.

The New York Times/Peter Yates

Dr. June Osborn
Dean of the school of public health at the University of Michigan

"The trends are awful. In the 25- to 45-year age group, everybody is going to know somebody suffering from AIDS."

But there is little evidence yet that the virus has been spread from one non-drug-using heterosexual to another through sexual intercourse. Thus far, the Centers for Disease Control says, the prevalence of infection in heterosexually active people with no known risk factor for AIDS in either partner is "very low on a national basis."

The broad-scale screening tests given to blood donors and applicants for military service have thus far been reassuring. The rates of infection among first-time blood donors and applicants for military service have remained stable for two years, at a fraction of 1 percent, indicating that the virus has probably not yet moved rapidly into the general population.

Condom Campaign Justified

Perhaps the earliest indication of heterosexual spread would be seen at venereal disease clinics, which typically treat the most sexually active individuals in a community, who are the most likely to become infected with the AIDS virus. Nine surveys at such clinics in six major cities found that only 0 to 2.6 percent of the heterosexuals were infected with the AIDS virus, as against 35 to 55 percent of the homosexuals attending the same clinics.

Still, officials warn that the potential for heterosexual spread of the virus remains, amply justifying campaigns to encourage condom use among those with multiple sex partners. Although homosexual men are by far the largest group of AIDS victims in the United States, more than a quarter of the victims are heterosexuals, of whom the largest group is drug addicts. These individuals did not acquire the disease heterosexually. But once infected, they are capable of passing it on to their sex partners, who if they are women can pass it on to the babies they bear.

In recent testimony to the President's AIDS Commission, Dr. Sheldon Landesman, director of the AIDS Study Group at the State University of New York's Health Science Center in Brooklyn, estimated that the pool of infected heterosexuals probably exceeds a quarter million people. He said "this is a large pool" of infected heterosexuals "no matter how you look at it."

"The impact of this population on future generations of women and children is just starting to be felt," he added.

A Worrisome Estimate

Although there is little hard evidence that the virus is being spread among heterosexuals who have had no contact with drug addicts or other risk groups, the Centers for Disease Control has made a very rough estimate on statistical grounds that about 30,000 heterosexuals without specific admitted or known risks may already be infected with the AIDS virus.

While they comprise a minute percentage of the heterosexual population, and are almost certainly concentrated in regions where drug addiction is common, these people are a potentially worrisome source of new infections.

Experts are split on how far the virus will spread among heterosexuals and on whether the epidemic will become self-sustaining in the heterosexual population. But nearly all agree that any invasion of the heterosexual population will be relatively slow.

"AIDS has caused a real fire in the drug community and the gay community," said Polly Thomas, who directs AIDS surveillance for the New York City Health Department. "When that fire burns out, it's not going away. But it's not going to explode in any other community in the same way."

New York Times - 2/14/88

Origin of Virus Is Murky, But Signs Point to Africa

The evidence is getting stronger that the AIDS virus first simmered in Africa before it exploded into a worldwide epidemic. But whether the AIDS virus is a relatively recent mutation or an ancient germ that has resided in some humans for tens of thousands of years is a mystery.

None of the evidence is conclusive. But most experts who have speculated on the origins of AIDS have focused on Central Africa as the likely incubator of the virus. No leading expert has mentioned any other continent as the likely origin, although some have questioned the evidence about Africa or suggested that the full explanation will never be known.

Scientists say that better knowledge of the history of the virus might provide clues in the search for treatments and vaccines, or lessons on how to curb future epidemics.

First Known Infection: 1959

Scientists who support the African hypothesis rely primarily on the fact that several other viruses that infect monkeys or humans in Africa are closely related to the AIDS virus, HIV-1, making it likely that the whole family of viruses may have evolved on the same continent.

Many also note that the earliest well-documented evidence of infection with the virus was found in Africa, in a blood sample taken in Zaire in 1959.

If Central Africa is the home of the virus, nobody knows how long it has been there or whether it came there from someplace else. Some theorists suggest that the virus is a relatively recent mutation from some related human or animal virus in Africa.

Other theorists believe the virus has existed in isolated villages for decades, centuries, or even for the entire history of the human race. They suspect that social changes, such as the migration from rural areas into the cities and an increase in sexual contacts may have allowed the wider transmission of the virus, which apparently began to spread in the 1970's. Then, with the increased ease of modern transportation, its spread around the world was virtually guaranteed.

How and when the virus first entered the United States is not known. It could have been introduced more than once, until a self-sustaining chain of person-to-person infection was established. Evidence suggests that the initial infections of the current epidemic began some time in the early or mid-1970's. The virus invaded the homosexual population at a time when promiscuity was a symbol of liberation for many gay men and mobility was easy, allowing its explosive spread.

son infection was established.

Tracking AIDS Through World Since '81

Since AIDS was recognized as a disease in the United States in 1981, it has appeared in three-quarters of the world's countries, about 160 nations in all, according to the World Health Organization.

Dr. Jonathan Mann, director of the special program on AIDS for the specialized United Nations agency, recently estimated that 150,000 cases of AIDS had occurred so far around the world.

The largest number of cases are believed to have occurred in Africa, although the disease is often unrecognized or unreported there. The United States, which has a vigorous surveillance system, has reported more than 50,000 cases, about a third of the world's estimated total.

Cases Appearing in Asia

The disease has established significant beachheads in Europe and in Latin America, from each of which more than 8,000 cases had been reported by the end of last year. But it has barely begun to appear in Asia, which had reported little more than 200 cases by the end of 1987. Although some Asian countries may not be recognizing or reporting all their cases, Dr. Mann said, "It seems clear the virus has only entered the Asian population relatively recently."

The worldwide toll is expected to climb rapidly. Dr. Mann predicted that there would be 150,000 new cases in 1988 alone, bringing the world total to 300,000 cases by the end of the year.

The picture could get even gloomier. The World Health Organization estimates that 5 million to 10 million people around the world are infected with the AIDS virus, which generally brings on acquired immune deficiency syndrome itself several years after infection. If that estimate is accurate, W.H.O. says, 500,000 to 3 million new cases of AIDS will emerge over the next five years in people already carrying the virus.

Patterns of Infection and Disease

Three broad patterns of infection and disease have been distinguished around the world. In North America, Europe, Australia and New Zealand, the virus has been present for several years and has primarily infected homosexual men, who were infected sexually, and intravenous drug users, who were infected through the sharing of needles.

In Africa and Haiti, the virus has also been present for some time, but the major mode of transmission appears to be heterosexual intercourse.

In parts of Africa the virus is still spreading through contaminated transfusions, since screening of blood donations is not yet universal.

In Asia, where the virus is still relatively rare, many infections have resulted from past exposure to contaminated blood products from industrial countries or sexual intercourse with infected foreigners.

AIDS: Who Is Infected?

Extrapolating from a variety of surveys, Federal officials recently made very rough estimates of the prevalence of infection with the AIDS virus in the American population.

	Estimated number in U.S.	Proportion infected with AIDS virus	Estimated number infected
Homosexual men	2.5 million	20-25%	500,000 - 625,000
Bisexual men and men with highly infrequent homosexual contacts	2.5-7.5 million	5%	125,000 - 375,000
Regular intravenous drug users (at least weekly)	900,000	25%	225,000
Occasional intravenous drug users	200,000	5%	10,000
Hemophilia A patients	12,400	70%	8,700
Hemophilia B patients	3,100	35%	1,100
Heterosexuals without specific identified risks	142 million	0.021%	30,000
Others, including heterosexual partners of people at high risk, heterosexuals born in Haiti and Central Africa, transfusion recipients	N.A.	N.A.	45,000 - 127,000
TOTAL			945,000 - 1.4 million

Source: Centers for Disease Control

CAMPAIGN TO FIND DRUGS FOR FIGHTING AIDS IS INTENSIFIED

AT LEAST 9 BEING TESTED

More Than 3,000 Patients Are Subjects in Studies by U.S. and Private Scientists

By PHILIP M. BOFFEY
Special to The New York Times

WASHINGTON, Feb. 14 — After a sluggish start that provoked criticism and bitter disputes, Federal and private efforts to develop new drug treatments for AIDS are accelerating.

In recent months, the Federal Government has screened thousands of chemical compounds in the laboratory to determine if they look effective against the AIDS virus, has established new drug development and testing units at major medical centers and has pushed several potential AIDS drugs into clinical trials in humans.

At least eight drugs that attack the AIDS virus and one drug that boosts the immune system are now being tested, either alone or in combination with other drugs, in trials involving more than 3,000 patients at the Government's network of testing centers. Another three drugs are expected to enter human trials in the near future, and two others are under negotiations for trials.

At the same time, drug companies are sponsoring, on their own, at least 15 controlled trials of experimental AIDS treatments that could enroll more than 2,000 patients in all.

Many With AIDS Virus

The drug developers are racing to find treatments that will benefit thousands of AIDS victims and a million or more people infected with the AIDS virus who have not yet become sick. Thus far, more than 52,000 people in the United States have contracted AIDS, and some 29,000 are already dead.

The Food and Drug Administration has now approved more than 100 Government and industry studies in humans of more than 40 different

AIDS: Confronting The Epidemic
Second of four articles.

drugs that either attack the AIDS virus or stimulate the body's immune system to attack the virus.

However, much of the drug testing is at very early stages in relatively few patients. For the foreseeable future, AZT, azidothymidine, the only drug that has already proven effective in prolonging the lives of victims of acquired immune deficiency syndrome, is expected to remain the chief treatment. It is made by the Burroughs Wellcome Company of Research Triangle Park, N.C.

The newly accelerated drug-development activity comes after a year of setbacks, start-up problems and delays as private companies slowly mobilized and the Government struggled to organize a network of drug testing units at 36 medical centers around the country. The sluggish start provoked a chorus of criticism from desperate patients and their advocates who sought prompt access to a wide variety of experimental drugs even though health officials insisted that few showed genuine promise and some were dangerous.

The recent surge in activity has not quelled the criticism. "Drug research

Continued on Page A14, Column 1

N.Y. Times
2-15-88

Research to Find Drugs for Treating AIDS Is Intensified

Continued From Page 1

is going at a maddeningly slow pace," said Dr. Barry Gingell, director of medical information for the Gay Men's Health Crisis in New York. "Right now at least 10 to 20 drugs show promising results elsewhere in the world or in the laboratory, but many potential therapies aren't being tested."

"Drug development has been very slow," said Dr. Mathilde Krim, founding chair of the American Foundation for AIDS Research. "We have tens of thousands of people sick with terminal disease in this country, and only 2,900 of them are in Government clinical trials. That's not very many."

Some Patients Skirt System

Dr. Krim said that the delays in beginning drug trials have forced many patients to "go around the system" and administer drugs to themselves without any guidance or proof of effectiveness and safety. "Thousands of people are taking different things, from macrobiotic diets to poisonous drugs, without knowing if it does any good," she said. "It's a very dangerous thing to do."

Even President Reagan's AIDS commission expressed concern in December that, after eight years of the epidemic, "there are so few drug therapies available for AIDS." In its preliminary report, the commission said that it "has decided to move immediately to investigate the lack of new drugs to treat AIDS patients." It will hold a three-day hearing on drug development and other issues in New York City later this week.

Dr. Frank Lilly, chairman of the genetics department at the Albert Einstein College of Medicine in New York who will chair the hearings, said that drug development efforts got off to a "very slow, very painful" start. But now, he said, "Progress has been made, there's no question about it. The situation is very much improved over a year ago, although there is still room for more progress."

Some physicians and scientists believe strongly that the Government should move more rapidly to test a wider variety of drugs in as many people infected with the AIDS virus as possible. "I think we know enough at this point to try various drugs in a very coordinated manner to see if we can stem this tide," said Dr. Michael Lange, an AIDS investigator at St. Luke's-Roosevelt Medical Center in New York City.

It's Not That Easy

Dr. Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, deplored the "misperception" that "if we're not testing every conceivable drug in a trial, we're falling short of our responsibility." All too often, he said, some scientist in Europe will throw a chemical into a test tube and report that it inhibits the AIDS virus, or some doctor will report that a patient got better after treatment. Soon, he said, "Everybody in New York and San Francisco is saying 'Why aren't you studying this? Thousands of people are dying in the streets, and this at least offers some hope. Why not try it?' "

But "it's not that easy," Dr. Fauci said, because when "scientifically qualified" people look closely at such claims, typically "there are no data." The Government has been reluctant to commit the nation's limited supply of clinical investigators to testing a series of dubious drugs, Dr. Fauci said. "Most of the time it is not worth going forward."

Initial Focus Was on AZT

The National Institute of Allergy and Infectious Diseases, which operates the Federal testing network, focused initially on expanding the testing of AZT. Federal officials argued that it made sense to rapidly expand knowledge of the only known effective drug before investigating others that looked far less promising. However, even many of the AZT trials that have already been started by the Government have been sluggish in enrolling patients. And critics note that AZT is so toxic that many ill patients cannot take it. They fault the Government for not moving more quickly with other drugs.

These criticisms are considered unfair by Federal health officials, who contend that they have moved with unusual speed to establish from scratch a complex testing network capable of producing reliable test data. Much of the criticism would dissipate, officials say, if people understood the full scope of the program.

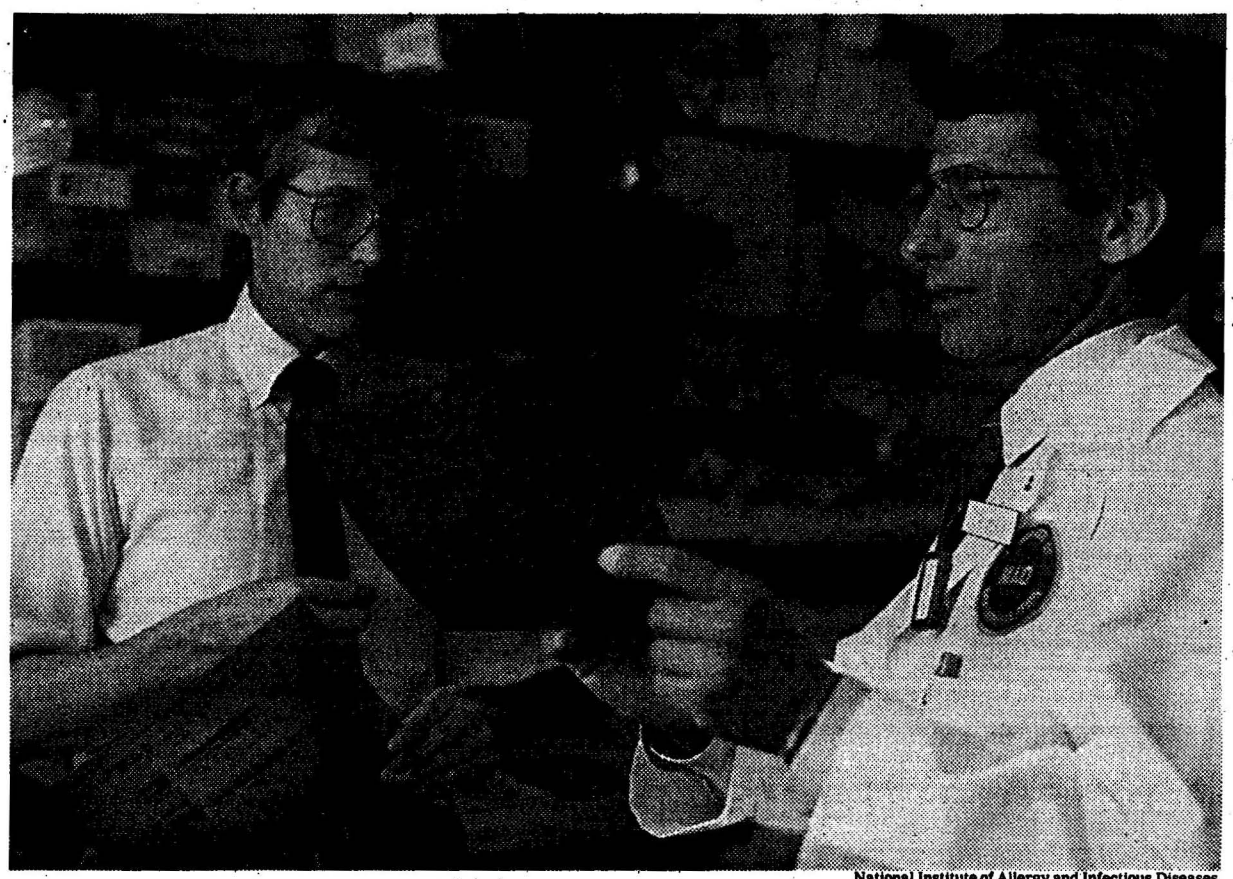
Over the past year, the Federal gov-

AIDS: The Search for Treatments

Most of the drugs below have been designated by Federal scientists as having the highest priority for research; the list does not include all the drugs involved in trials. Other drugs are listed because they have received great public attention and in some cases are being taken by people who have obtained them through a variety of channels outside the controlled trials.

Drug and Manufacturer	Action	Federal Priority	Status
AL-721 Ethigen	Antiviral	Low	Small-scale trials inconclusive. Federal effort to measure effect of different doses now enrolling patients.
Ampligen HEM Research, Du Pont	Immunity stimulant, possibly antiviral	Medium	Company-sponsored trial still enrolling patients; Federal study of dosage levels due to start shortly.
Azidothymidine (AZT) Burroughs Wellcome	Antiviral	High	Only federally approved anti-AIDS drug, already used to treat more than 19,000 patients worldwide. About 40 clinical trials now exploring effectiveness in different patient groups and in combination with other drugs.
Dextran sulfate Ueno Fine Chemicals	Antiviral	High	Long used safely as an anticoagulant. Company-sponsored study of dosages under way and Federal trial under development.
Dideoxycytidine (DDC) Hoffmann-La Roche	Antiviral	High	More powerful than AZT in laboratory; human trials delayed to find solution for toxic side effects.
Foscarnet A.B. Astra	Antiviral	High	Federal study of effective doses now enrolling patients. Clinical studies in Europe nearing completion.
GM-CSF (colony stimulating factor) Immunex, Genetics Institute, Sandoz, Schering-Plough	Immunity stimulant	High	Company-sponsored studies under way; Federal trials in combination with other drugs being planned.
HPA-23 Rhone-Poulenc	Antiviral	Medium	French results inconclusive. Federal Government negotiating with manufacturer for new trial.
Imuthiol (DTC) Merieux Institute	Immunity stimulant	Low	Company-sponsored trial near completion.
Interferon alpha Burroughs Wellcome, Hoffmann-La Roche, Schering-Plough	Immunity stimulant and antiviral	High	Company-sponsored trials show benefit against Kaposi's sarcoma. Federal trials in combination with AZT under way.
Interleukin-2 Cetus, Hoffmann-La Roche, Immunex	Immunity stimulant	High	Federal pilot study in combination with AZT now enrolling patients. Small study of IL-2 alone showed no benefit.
Ribavirin ICN Pharmaceuticals	Antiviral	High	Company-sponsored clinical trials flawed and inconclusive. Federal trials about to start.

Compiled from Government and industry sources and the American Foundation for AIDS Research



National Institute of Allergy and Infectious Diseases

Dr. Anthony Fauci, right, director of the National Institute of Allergy and Infectious Diseases, and a colleague, Dr. H. Clifford Lane, looking at an AIDS test result. Dr. Fauci deplored the "misperception" that "if we're not testing every conceivable drug in a trial we're falling short of our responsibility."

ernment has greatly increased its capacity to screen drugs in the laboratory. The National Cancer Institute is directing a new program in which cells in the laboratory are infected with the AIDS virus and chemicals are added to see their effects. The system has already screened thousands of drugs at a rate that will exceed 10,000 a year, according to Dr. Bruce A. Chabner, director of institute's division of cancer treatment. A few promising candidates have emerged, but these are far from ready for trial in humans.

The Government has also organized 18 cooperative drug discovery groups, involving researchers from academic, industry, and Government centers, that will seek to design new compounds against the AIDS virus based on the improving intricate knowledge of the virus. Most of the early drug work simply examined compounds that were already available, such as AZT. But some feel that the design of drugs to attack known vulnerable points in the life cycle of the virus holds the greatest long term promise. "Whether we can do a lot better than AZT by random screening is an open question," said Dr. David Baltimore, a Nobel Prize-winning molecular biologist. "We may have to look for more rational drug development."

In one pioneering approach, reported in December, scientists used genetic engineering techniques to synthesize copies of a protein, CD-4, that was able, in the laboratory, to sop up the AIDS virus like a sponge. However, any potential clinical applications are years away.

Clinical Trials Expanded

The Government has recently expanded its human clinical trials of drugs that show potential in the laboratory or in animals. The National Institute of Allergy and Infectious Diseases initially concentrated on testing AZT in different categories of patients and in combination with other drugs at 19 Aids Treatment Evaluation Units at major medical centers. In recent months it has meshed another 17 clinical research teams into the network and has pushed several more drugs into at least small-scale trials.

The network has begun testing, either alone or in combination, the antiviral drugs DDC, or dideooxycytidine, which was developed by the Federal Government and licensed to Hoffmann-La Roche Inc. of Nutley, N.J.; Foscarnet, made by A. B. Astra of Sweden; AL-721, made by Ethigen Corporation of Los Angeles; alpha interferon, made by Burroughs Wellcome, Hoffman-La Roche and Schering-Plough Corporation of Madison, N.J.; gamma interferon, made by Genentech Inc. of San Francisco; acyclovir, made by Burroughs Wellcome, and tumor necrosis factor, made by Genentech.

It will soon test the antivirals ribavirin, made by ICN Pharmaceuticals of Costa Mesa, Calif.; ampligen, made by HEM Research of Philadelphia and E. I. duPont de Nemours and Company of Wilmington, Del., and granulocyte-macrophage colony stimulating factor, made by the Genetics Institute of Cambridge, Mass., Immunex Corporation of Seattle, Sandoz Pharmaceuticals Corporation of East Hanover, N.J., and Schering-Plough. The Government is negotiating with Rhone-Poulenc Pharmaceuticals of Monmouth Junction, N.J., to test the antiviral HPA-23.

Among the immunity stimulants, it is already testing interleukin-2, made by Cetus of Emoryville, Calif., Hoffmann-La Roche and Immunex, and is negotiating with Newport Pharmaceuticals of Newport Beach, Calif., to test isoprinosine. In some cases, industry is already sponsoring human trials of the same compounds.

Final Authority Is Transferred

The decision on which drugs to test had been made until recently by Dr. Fauci, who relied heavily on the recommendations of a 19-member committee composed of top scientists from the Government and the medical centers that do the testing. The committee reviews the scientific evidence available on the candidate drugs and assigns a high, medium, or low priority for testing, or advises against testing at all. Two months ago, the final authority for approving clinical trials was transferred from Dr. Fauci to committees representing the testing centers and the National Institutes of Health.

The rating committee's expert judgments often conflict with the rumors of a drug's effectiveness circulating among AIDS patients or generated by press coverage. Thus, HPA-23, the drug that Rock Hudson flew to Paris to obtain in a vain attempt to save his life, was rated only a medium priority by the committee. AL-721 and Imuthiol, two drugs that are highly popular on the underground market, were rated low priority, as was Peptide T, made by Peninsula Laboratories of Belmont, Calif., a drug that has received wide attention in the press.

As the clamor for new treatments has mounted and many AIDS patients have resorted to drugs obtained on the underground market, officials have relented from their priority ratings and begun human testing of AL-721, a substance that is highly popular with patients because it is easy to take, seemingly nontoxic, and can be made at home from widely available recipes. Few if any officials expect the substance to prove effective, but they contend that a trial is needed because so many people are using it.

Even the drugs that have been ranked high in priority are not considered particularly promising by many investigators. "Most of the agents deserve testing based on what's been reported, but the testing will be done without any sense of enthusiasm that they'll prove extremely active," said Dr. Paul Volberding, a top AIDS investigator at San Francisco General Hospital, who is a member of the Federal rating committee. "The big problem now is the lack of any very exciting compounds after AZT."

Caused Pains in Feet

"A number of things that are not likely to be useful are going into early testing," said Dr. Roy Widdus, former coordinator of AIDS activities at the National Academy of Sciences, who has just joined the staff of the World Health Organization. "There's a paucity of things that are worth testing."

"AZT is still overwhelmingly the most effective drug," said Dr. Fauci. "There isn't anything approaching it except maybe DDC." Although DDC proved too toxic in its initial human trials, causing disabling pains in the feet, investigators reported last month that alternating AZT and DDC on a weekly basis reduced the toxicity of each drug, offering hope that DDC can be used in effective treatments.

Among the other drugs, the one that most excites Dr. Fauci is interferon alpha, a drug that has received relatively little public attention as an AIDS treatment. Under certain circumstances, Dr. Fauci said, he would actually prefer treatment with interferon alpha to treatment with AZT. Thus, if he were infected with the AIDS virus and his only major clinical ailment were Kaposi's sarcoma, a cancer, Dr. Fauci said that he would seek treatment with interferon alpha alone because it has been shown effective in reducing Kaposi's in some experiments and is far less toxic than AZT. But if he had another opportunistic infection as well as Kaposi's, he would take a combination of AZT and interferon. And if he had AIDS without Kaposi's, he would take only AZT, he said.

The task of designing new compounds is intrinsically difficult because medical science has had few successes in finding drugs that are effective against viruses, in contrast to the vast array of pharmaceuticals that attack bacteria, parasites, funguses and other microbes that infect humans. AZT is one of only seven antiviral drugs on the market.

"The real problem is, where do you get the ideas and where do you get the compounds from?" said Dr. Frank Young, commissioner of the Food and Drug Administration. "That's been a major block. I feel the frustration and the terror. But the real hang-up is the science. We're dealing with a diabolica infectious agent."

From Infection to Illness: A Virus's Advancing Toll

Special to The New York Times

WASHINGTON, Feb. 14 — Individuals who are infected with the AIDS virus but are not yet sick face an ever-increasing risk that they will develop symptoms of the disease as the years go by.

Officially, the Federal Government estimates that 20 to 30 percent of those infected with the virus will develop AIDS within five years.

But several studies of blood samples from AIDS victims reveal that the risk keeps rising in subsequent years, with great variation among individuals.

In one group of 155 homosexual and bisexual men in San Francisco whose date of infection is approximately known, scientists found that very few developed AIDS in the first two years. But 5 percent had developed AIDS after three years, 10 percent by four years, 15 percent by five years, and 24 percent by six years.

After seven years and four months, 36 percent had progressed to AIDS. An additional 40 percent had other signs or symptoms of infection, such as oral funguses, prolonged fever, or severe weight loss. Only one in five remained completely free of symptoms.

Long-Term Questions

Scientists are unsure whether progression to disease in this group reflects the general experience of most people infected with the virus. But other studies have estimated that about 30 percent of hemophiliacs infected with the virus through contaminated blood plasma products develop AIDS within 6 years of infection, and that about 25 percent of those who received the virus through contaminated blood transfusions develop AIDS within five years.

The big question is whether the percentage of infected individuals who become ill keeps rising or eventually tapers off.

"The final toll is unknown," said Dr. Anthony Fauci, director of the National Institute of Allergy and Infectiouse Diseases. "Will virtually everyone who is infected develop full-fledged AIDS after 30 or 40 years? Or will it plateau at 35 to 40 percent?"

Dr. Fauci recently told the President's AIDS Commission that evidence from the Walter Reed Army Medical Center suggests that 80 to 90 percent of all infected individuals experience some level of deterioration in their immune systems over a few years, suggesting that the vast majority of them "will be adversely affected by the virus over time."

Nobody has determined whether infected individuals can improve their chances of staying healthy by abandoning the practices that may have infected them, such as sexual relations or sharing need les with others who are infected, or by eating particularly nutritious foods, avoiding fatigue and stress, and doing everything possible to avoid additional illness or infections.

Many doctors recommend such practices on the theory that any additional germs or stresses may cause the AIDS virus, which resides within the immune system, to accelerate its lethal destruction.

Large-scale studies are under way to determine whether AZT, or azidothymidine, a drug that has already been shown to prolong the survival of patients with full-fledged AIDS, can also slow or prevent the the development of AIDS in individuals who are infected but not yet sick. Other drugs are also being investigated for this purpose.