RethinkingAIDS

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IS EVERYBODY POSITIVE FOR HIV? Is anybody infected by HIV?

Professional HIV-testing expert Roberto Giraldo, MD, noticed something strange. Whereas HIV-antibody test procedures stipulate very high dilutions of patient sera, instructions for other viral antibody tests call for little or no diluting. Giraldo examined 83 subjects with sera that, when heavily diluted as directed, tests HIV-negative. But when undiluted, all the sera tested positive.

What could this mean?

by Paul Philpott

PHYSICIAN Roberto Giraldo realized something didn't fit soon after he began working at a prestigious New York City university hospital laboratory that runs tests for a variety of microbes. The HIV-antibody test instructions call for technicians to heavily dilute patient sera (cell-free blood similar to plasma). The antibody tests for all other viruses call for little or no diluting.

What justified this extraordinarily large amount of diluting? Giraldo asked colleagues and lab technicians, sent e-mails around the world, phoned test company representatives, and performed rigorous literature searches. Yet he found no answers. Worse, nobody found his question in the least bit interesting — except those who reject the HIV explanation of AIDS. But even they had no answers.

Giraldo wondered what would happen if he evaluated patient sera that tested HIV-antibody negative when diluted the unusually large amount as instructed. What if he treated such sera according to normal antibody testing standards? In other words, what if he tested officially HIV-antibody negative patients using undiluted sera? Would the sera that were negative when diluted be positive when undiluted? His research revealed that nobody had yet examined such questions. So he tried it himself.

According to a technical paper he wrote for the midwinter 1998-1999 *Continuum* (www.virusmyth.com), an AIDS reappraisal magazine, Giraldo tested undiluted sera from 83 officially HIV antibody-negative patients. To his astonishment, every one of the undiluted sera tested positive. These findings, Giraldo says, represent yet another fatal paradox for the HIV explanation of AIDS.

Giraldo's background

Girlaldo is an expert in internal medicine and infectious diseases. He received his MD in his native Colombia, and a masters degree in infectious diseases from the University of London (*RA* April 1997). He is also a former biology department chairman at a large Colombian medical school. For the last six years he has worked in a clinical immunology laboratory at a major university medical school in New York City. (*RA* will withhold the university's name to protect Giraldo from the usual professional repercussions experienced by those who express scientific conclusions that cast doubt on the highly popular and financially profitable HIV explanation of AIDS).

Giraldo's daily responsibilities include performing the tests used for diagnosing HIV status; namely, ELISA and Western blots that detect antibodies that neutralize presumed HIV proteins, and the dubious "viral load" test that detects and amplifies trace numbers of tiny portions of the presumed HIV genome.

Giraldo has long doubted the validity of these tests, and contested the official interpretation that positive results indicate HIV infection. He considers it unjustified to diagnose HIV infections using these tests.

High dilutions tipped off Giraldo

"The extraordinarily high dilution of the person's serum — 400 times for the ELISA and 50 times for the Western blot — took me by surprise when I first learned to administer them," Giraldo says. "Most serologic tests that look for the presence of antibodies against germs use undiluted serum, called 'neat,' or 'straight.' For example, the ELISAs that look for antibodies to hepatitis A and B, rubella, Histoplasma and Cryptococcus viruses, and syphilis bacteria, to mention just a few, use straight serum.

"However, the ELISAs for antibodies against some germs do require slightly diluted serum. For example, ELISAs that look for antibodies to measles, varicella, and mumps viruses use a dilution of 1:16, to cytomegalovirus (CMV) 1:20, and to Epstein-Barr Virus (EBV) 1:10." Presumably, these slight dilutions improve the accuracy of positive results in identifying people who really do have active infections, and of negative results identifying people who really do lack active infections.

With the HIV tests, though, no isolation data exists to justify or explain the dilution levels.

"For years I searched the medical literature and studied the manufacturer's documentation to find the reason for these very high dilution requirements," Giraldo says. "I even phoned representatives of the test manufacturers. The most confident responses I received were that, 'The tests were standardized that way.' That leads

me to conclude that only the members of Robert Gallo's NIH lab who devised these tests and introduced them in 1984 (*Science*, May 4) can answer the question: why dilute?"

"I also began to question the terms 'positive' and 'negative' used to describe the results of antibody tests," he recalls. "Anyone who performs these tests for any microbe or other antigens knows that the results are not like a light bulb, on or off. Some people's serum reacts slightly, but not enough to earn the 'positive' designation. And among those whose serum reacts strongly enough to earn the 'positive' designation, some react stronger than others."

Giraldo searched the medical literature to find the rationale for the HIV ELISA and Western blot testing procedures.

Although isolation studies establish the testing procedures for other viruses, Giraldo found no isolation data at all for HIV. Nor did he find any other justification for the mysteriously high serum dilution levels, or, for that matter, the luminosity level used to declare a reaction positive, the array of protein reactions that constitute a Western blot positive designation, or the non-use of HIV antigen tests.

His studies uncovered the work of the Australian research team led by biophysicist Eleni Papadopulos-Eleopulos. Eleopulos has searched extensively for isolation data that justifies the HIV tests, but has found none (*RA* June/July/Aug. 1997). Her work inspired another researcher, virus isolation pioneer Etienne de Harven, to closely examine the issue. He concurs with her (*RA* Nov./Dec. 1998).

Giraldo's experiment

"My curiosity led me to conduct an experiment in a medical laboratory in Yorktown Heights, New York. First, I took samples of my own blood, which, at the mysteriously stipulated 1:400 dilution, reacts negative. I then ran the exact same serum samples through the test again, but this time at 1:1 [undiluted]. Tested straight, my sera reacted positive every time.

"Next, I tested the undiluted serum of other subjects whose heavily diluted (as stipulated by the instructions) serum tests HIV-negative, just like mine. I obtained the serum of 83 officially HIV-negative subjects. I confirmed that at the high stipulated dilution level, each sample tested negative. But when tested straight — undiluted — every sample tested positive, just like mine.

"I should mention that with the exception of my own blood, the patient samples all came from doctors who requested HIV tests. According to my experience, this usually this means that the patient belongs to one of the official AIDS risk groups [gay men and drug injectors]."

Giraldo also considered the amounts of antibodies that the test results indicated. "According to the Abbott Laboratory documentation," he says, "the absorbance value [yellow color intensity] develops in proportion to the amount of antibodies to HIV-1 which are 'bound to the bead.' I noticed that the absorbance values of the specimens that tested negative when diluted [1:400], but positive when undiluted [1:1], had lower absorbance values than the samples that, diluted the specified amount, react positive on both the ELISA and Western blot tests. This probably means that the blood that tests negative when diluted but positive when undiluted has a lower level of antibodies than the diluted blood that tests doubly positive."

So, all people, it seems, may have some amount of "HIV antibodies" in their blood. And therefore, to some extent, everybody

ELISA and Western blot testing

Appreciating Giraldo's conclusions requires an understanding of the ELISA and Western blot techniques.

Both ELISAs and Western blots detect proteins, either antibodies or antigens. Antigens are foreign proteins, such as those belonging to viruses, which the immune system targets for destruction. One way that immune systems destroy antigens is by producing antibodies that lock onto, or "neutralize," them. A particular virus may contain about ten different proteins that get exposed to the immune system. Each one of these proteins triggers the production of different species of antibodies, just as a door with ten bolt locks requires ten different keys.

ELISA stands for Enzyme-Linked Immunosorbent Assay. Special enzymes linked to the test proteins luminate at intensities according to the amount of targeted protein that exists in the serum. Western blots work similarly. But, unlike ELISAs, which hold the different species of test proteins together, Western blots separate the different test protein species into different bands, according to their molecular weights. Thus, whereas a positive ELISA indicates that serum contains target proteins that react with at least one test protein, it cannot determine how many or which species of test proteins have reacted. Western blots can.

(The word "Western" honors the scientist who developed the technique, first used for DNA. The DNA procedure is called the "Southern blot," after the scientist's last name, and when employed for RNA, the procedure is called the "Northern blot.")

These tests are inexpensive, easily performed substitutes for the only absolute method of determining if a person is actively infected with a microbe: isolation of the microbe from fresh patient tissue. In the case of a microbe that infects immune cells, which is what HIV is said to be, that would mean isolating HIV from fresh blood.

The accuracies of these tests are determined by successfully obtaining positive results in people from which isolations can be obtained (sensitivity), and successfully obtaining negative results in people from which isolations cannot be obtained (specificity).

Establishing test parameters

Though reactivity of a single target protein earns a positive ELISA designation, a positive Western blot may not require that every target protein react—not if microbial isolation data shows that a certain combination of positive reactions corresponds to a maximum accuracy in identifying people who are and who are not actively infected

may be "HIV-positive." What could this mean?

The implications

Using the officially stipulated serum dilutions, very few Americans test positive for antibodies that neutralize presumed HIV proteins (*RA* July, 1996). Among Americans in general, only about one in 260 test positive. That number plummets to just one in 7,500 if risk group members are excluded. Only when the risk group members are considered exclusively does the number become appreciable. About half of all gays and drug injectors in large cities test positive, as do 75% of all hemophiliacs (*RA* Nov. 1997). And 10-20% of the general populations of various African countries reportedly test positive.

The figures are even higher for risk group members who develop any of the diseases that compose the official AIDS definition. Among a mixture of gay men and African heterosexuals with these diseases, 88% test positive according to Gallo's original 1984 data (*Science* May 4). More recently, data analyzed in 1995 by UC-Berkeley retrovirologist Peter Duesberg (*Genetica* 95) showed that 82% of gay men with these diseases test positive.

With his data suggesting that perhaps all people may have varying amounts of "HIV antibodies" in their blood, Giraldo has a reasonable explanation for how Gallo may have established the ELISA and Western blot HIV testing standards: they happened to correspond with high success in identifying members of the AIDS risk groups, especially those who have AIDS diseases, while distinguishing them from people unlikely to belong to the risk groups or to have AIDS conditions.

By heavily diluting the serum prior to testing, and using a particular luminosity level as official standards dictate, positive results occur only for people who possess very high levels of these antibodies. But undiluted serum will react positively even for people who test negative when their serum is diluted as specified. Giraldo hypothesizes that different people's sera would react as positive according to different amounts of dilution. People with large amounts of these antibodies would react positively even with the very high dilution rates stipulated by the standard instructions. Other people would have only enough of these antibodies to cause a reaction if their serum was diluted some intermediate amount. Others with very low levels of these antibodies, may produce positive test results only if their serum is not diluted at all.

Gallo's team designed and patented these tests in order to identify people who have, or who are likely to have, AIDS diseases. Gallo presumed — but did not prove — that these tests would also indicate infections with a common virus that caused these diseases. Gallo's team settled on testing standards that produced positive results in 88% (43 of 49) of his risk group test subjects who had AIDS diseases, 79% (11 of 14) of his risk group test subjects who had "pre-AIDS," 40% (9 of 22) of his risk group subjects with no AIDS conditions, and less than 1% (1 of 164) of AIDS-free test subjects who did not belong to the official risk groups.

That means Gallo's antibody test battery — the same used today to determine "HIV status" — has some reasonable accuracy in identifying people who belong to AIDS risk groups, especially those who have AIDS conditions. But no data establishes any accuracy of this testing battery for identifying people who have infections with any particular virus.

According to Giraldo, Eleopulos, and de Harven, researchers

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with that microbe.

Giraldo emphasizes a point that most HIV professionals overlook, and which plays a salient role in his investigation: ELISAs and Western blots are not only qualitative (they indicate if the target proteins are present in the serum), they are also quantitative (they indicate how much of the target proteins are present in the serum). Each can measure the amount of target proteins in the sera by the intensities of the test reactions, as determined by their observed luminosities.

ELISA and Western blot test instructions stipulate what luminosity level constitutes a positive reaction, and that the level varies according to the microbe being tested for. That opens the question: At what level of luminosity should a reaction be regarded as "positive"? The answer, as always, lies with isolation of the microbe. Only isolation data can logically determine the ELISA and Western blot luminosity levels that most accurately distinguish who has or doesn't have an active infection with a particular microbe.

ELISAs and Western blots can test for either antigens or antibodies, depending on what the test kit contains. Antigen tests contain antibodies and react if the serum contains antigens (the actual virus proteins, in the case of a viral test); antibody tests contain antigens and react if the serum contains antibodies. The HIV antibody tests, then, contain presumed HIV proteins, which are the viral antigens. They react with sera that possess antibodies that neutralized these antigens. What is called "the HIV test" consists of a battery of sequentially administered antibody tests, two ELISAs followed by at least one Western blot.

Both antibody and antigen tests can be reliable and valid indicators of viral infections. But only virus isolation can demonstrate if either accurately identifies who has and who doesn't have an active viral infection.

ELISA and Western blot tests exist for HIV antibodies and for HIV antigens. But the HIV antigen tests are not used for diagnosing HIV infections. Like the questions Giraldo has asked about the unusually high dilution levels for HIV tests, nobody has ever explained why HIV-antigen tests are not used to diagnose HIV infections. But the technical literature is very clear: while many members of the risk groups, including most who have "AIDS" conditions, test HIV-antibody positive, only those with “AIDS” conditions tend to test HIV-antigen positive as well (Piatak, *Science* 259, 1993). So whereas HIV antibody tests identify as positive lots of healthy people, antigen tests do not.

Giraldo says that even diluting of serum can be a valid practice and produce reliable results. But, again, only if the diluting has been established by isolation to improve accuracy.

— *P. P.*

have failed to determine a success rate for isolating a viral species from people who test positive on the HIV ELISA and Western blot antibody tests.

Thus, in terms of these tests being used as they are to identify people with HIV infections, Giraldo concludes that there is no valid justification for the high dilution levels, for the luminosity criteria for determining positive reactions, for favoring antibody tests over antigen tests, or for the array of reactions that qualify a Western blot as positive.

Further experiments and viral load

Giraldo acknowleges that many important questions remain unanswered. For example, he did not examine the HIV Western blot or "viral load" tests.

"Lacking any funding to support this research," Giraldo says, "I have only been able to examine the HIV ELISA, and not as thoroughly as I would like. Because the Western blot HIV tests use the same proteins as does the ELISA HIV tests, and also requires an unusually high dilution — although only 1:50—I expect the same results if I similarly examined it. However, I have not had the opportunity to check this hypothesis. I hope to raise the money to examine the HIV ELISA more closely, and to examine the HIV Western blot antibody test using the same process.

"Also I would like to examine the HIV viral load test," which also involves diluting, and other important paradoxes as well (*RA* Oct. 1996). Chief among them: like the HIV ELISA and Western blot tests, the viral load test accuracy has not been established using the only valid method — viral isolation.

As far as Giraldo can tell, the viral load test was invented especially as a way of artificially demonstrating large amounts of HIV RNA, when conventional methods accurately establish that there is little or none present.

Non-HIV explanations in order

With no HIV isolates documented in the literature, and Eleopulos as well as others having shown that AIDS distributes epidemiologically unlike a contagious condition (Duesberg, *Inventing the AIDS Virus*), Giraldo looks beyond a viral explanation to understand positive reactions on the "HIV tests." He refers to the work of Eleopulos. The purported HIV constituents from which the "HIV tests" are derived seem to her to be ordinary constituents of the human constitution. This would seem unlikely had the "HIV material" used in the tests been extracted from HIV isolates — that is, from samples that consist entirely of virus-looking objects determined to behave like viruses. But Eleopulos found that all samples presented in the medical literature as "HIV isolates" consist mostly of clearly non-viral material, mixed in with a minority population of objects labeled as HIV. And those objects, Eleopulos contends, fit the description of ordinary cellular "microvesicles," not viruses. And she finds no data that precludes any of the "HIV isolate" material from being normal cellular constituents. The retroviral pioneer de Harven agrees with this assessment.

It seems to Giraldo, then, that the HIV-antibody tests indicate exposure to factors that increase the production of antibodies that react with proteins found in samples mislabeled "HIV isolates." These might include an array of factors identified by Eleopulos and others as the likely causes of AIDS: the consumption of narcotics, hemophilia treatments, transfusions and the conditions that make them necessary, and the various aspects of Third World poverty.

He has no preliminary hypothesis for what the "viral load" test might indicate because he has not yet examined it.

The experiments that he proposes would help explain what these tests mean. One thing is already certain: the existing data do not confirm the hypothesis that positive HIV tests of any sort indicate infection with any species of virus. Giraldo contends that everybody may produce some level of antibodies against the presumed HIV proteins. But he has no reason to conclude that anybody harbors an HIV infection, HIV positive or not.

Rethinking AIDS

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