Questioning the Test Related Articles

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Testing, Testing… Is a Positive Antibody Test Proof of HIV Infection?

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“An antibody test, even if repeated and found positive a thousand times, does not prove the presence of a viral infection. The failure to verify the antibody tests against the gold standard of virus isolation is a serious omission of scientific method…”

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What evidence authenticates a positive HIV antibody test as proof of HIV infection? This question has greatly interested me since those of us working in Emergency Medicine spend a considerable part of our lives exposed to other people's blood and body fluids, a circumstance that according to the experts places us under constant threat of AIDS. If the experts are right, the life we save may cost us our own. Given this ironic and sobering circumstance, some of us have pursued the question of proving HIV infection to the very limits.

From the early days of AIDS I was fortunate to collaborate with Eleni Eleopulos, a Biophysicist at the Royal Perth Hospital, John Papadimitriou, Professor of Pathology at the University of Western Australia, and other colleagues. In one of our papers published in June 1993 in the journal Bio/Technology [1], we were compelled to confront many unsettling conclusions about the HIV antibody tests, none of which accord with current wisdom, and some of which I share in this article

The HIV antibody tests do not detect a virus. They test for any antibodies that react with an assortment of proteins experts assure us are unique to HIV. As almost everyone agrees, HIV is a retrovirus and the cause of AIDS [2].

When you take an HIV antibody test, what happens is this: A sample of blood serum is incubated with a mixture of these proteins in a test called an ELISA, an acronym for Enzyme Linked Immunosorbent Assay. The ELISA is positive if the serum solution changes colour. A color change indicates a reaction between the proteins in the test kit and the patient's antibodies. However, according to many experts, the ELISA is not specific, meaning it may react in the absence of HIV infection.

In response to this, testing authorities have developed strategies such as repeat testing of all positive ELISAs and following up those twice positive with a third but different antibody test known as the Western Blot. In the Western Blot, the "HIV proteins,” about ten of them, are located at discrete spots in a paper strip rather like the one your doctor uses to perform multiple tests on your urine. Serum is added and wherever there is a reaction a colour change occurs which shows up as a dark band. The test is read by noting which bands show up, in other words, which proteins react. Certain combinations of bands are defined as a positive test.

It is enigmatic that the location and number of bands required for a positive Western Blot varies around the world. They may even vary between laboratories within the same city. In Australia, four bands are required. In Canada and much of the United States, three bands suffice, while in Africa, two will do. In the US Multicenter AIDS Cohort prospective study involving several thousand gay men, just one "strong band” was deemed sufficient to earn a positive result.

If each of the above criteria indicates HIV infection, then HIV must cause different populations of antibodies to appear in different places. I don't know about you, but to me that sounds very odd. At the least it gives some Africans a way out. All an African has to do is have a test in Australia because two bands would not be considered positive here. Nevertheless, in spite of lack of standardization and other problems such as reproducibility, the Western Blot is accepted to be in excess of 99.9% specific and if positive is regarded as synonymous with HIV infection. In some countries, similar claims are now made for the HIV ELISA without “confirmation” by Western Blot.

The rationale for the use of antibody tests is as follows: The immune system has the ability to detect foreign agents and to respond by producing antibodies that react with those agents. However, this does not work in reverse. By that I mean the observation of an antibody reaction with a particular agent is not automatic proof that the antibody was produced in response to that agent.

The problem is that antibodies indulge in casual and indiscriminate relationships. They are in fact promiscuous. Antibodies meant for one agent may react with another agent, a perfect stranger. Or, if you want it put technically, there is ample evidence (some of the best in fact comes from the Pasteur Institute) that antibody molecules, even the most pure (monoclonal antibodies) are not monospecific and cross-react with other, non-immunising antigens.

Here are some examples to illustrate this most crucial fact. Firstly, in a study of 1.2 million applicants for US military service [3], of the 1% or 12,000 who had first time positive HIV ELISAs, only 2000 were ultimately shown to be also WB positive and thus, according to the authors, HIV infected. That left 10,000 positive ELISAs which must have reacted for reasons other than "HIV antibodies", a fitting testimonial to the problem caused by cross-reacting antibodies.

Secondly, there is the tantalising data reported in 1990 about dogs. Writing in the journal Cancer Research, Strandstrom and colleagues reported that 72/144 (50%) of dog blood samples "obtained from the Veterinary Medical Teaching Hospital, University of California, Davis" tested in commercial Western Blot assays, "reacted with one or more HIV recombinant proteins (gp120--21.5%, gp41--23%, p31--22%, p24-- 43%" [4]. Assuming California dogs are not

infected with HIV (as did the authors), one must conclude these data are further proof of antibody cross reactivity to many of the "HIV proteins."

What all this means is that you're not necessarily infected with what your antibodies appear to tell you. This can be brought home by two further examples. Firstly, some AIDS patients have antibody reactions with laboratory chemicals but no one claims AIDS patients are infected with laboratory chemicals. Secondly, as an example removed from AIDS, the antibody test for glandular fever relies on the fact that patients with glandular fever make antibodies that react with the red blood cells of sheep and horses. But these patients are not infected with animal blood and animal blood does not cause glandular fever. Bearing all these examples in mind it is painfully obvious we cannot pronounce someone infected with what is regarded as a lethal human retrovirus merely because we observe an antibody reaction. Before we pronounce any such reactions indicative of HIV infection and long before we introduce the test into routine clinical practice, we must exact solid proof of precisely why these reactions take place. In doing we must not forget that biology is not mathematics and despite our clever technology, in biology still we must stoop to the relative ignominy of empirical proofs. Or, as Plato said, "experiential data must always be interpreted in the light of what Nature has revealed".

In science, we must constantly resist the temptation to stray beyond our data, and in that spirit I put it to you there are only two pieces of information that can be gleaned from an antibody test (for mathematical purists, it's only one piece of information). Either you see a reaction or you don't. That's all. You don't see antibodies with labels attached saying what produced them. You cannot construe the genesis of antibodies by observing changing colours in a test-tube. The cardinal problem scientists face when ascribing meaning to a set of antibody reactions is how can they tell whether the reaction is caused by a real antibody or an imposter? One whose proper partner is something else? In this context it is proper for a disinterested scientist to allow for the possibility that there are no real HIV antibodies whatsoever, that they're all pretenders.
When the only information is a reaction, and that reaction has more than one possible cause as is the case with an antibody test, you need extra information before you can ascribe a particular outcome. So, if you want to claim an antibody reaction signals a particular outcome such as HIV infection, first you have to prove it. And just before we get to crunch time, consider this little morsel: AIDS patients are exposed to many foreign agents are known to have antibodies reacting with dozens of different substances and it makes perfect sense that the more antibodies there are the more chance there will be some that will spoil the test. What this means is that in the very patients you suspect of harboring a virus there exists the precise circumstances, lots of potentially cross reacting antibodies, which make it imperative to sort out what is really going on.

What's the solution or, more to the point, what's the problem? The problem is how do you know when you witness an antibody reaction, that is, a positive test, that HIV itself is present, too? After all, that's what you really want the test to tell you and the question on the patient's lips is bound to be "Is HIV infection the only cause of a positive test? If it's something else I'd rather have that, thank you very much". In technical terms the patient's hopes are hanging on the specificity of the test.

Let me explain specificity and what is meant by 100% specificity. One hundred per cent specificity means that positive tests only occur in HIV infected people. That's the same as saying positive tests never occur in uninfected people. And that's the same as saying all uninfected people have a negative test. This leads us tothe formal, mathematical definition of specificity. Specificity is the number of negative tests in a large group of individuals who do not have HIV infection. If 100% of one thousand people who do not have HIV infection test seronegative, the specificity is 100%. If one uninfected person tests seropositive, the specificity is reduced to 999/1000 or 99.9% by virtue of a lone false positive. Thus, to determine the specificity of an antibody test we need two pieces of data. The numbers of persons with negative tests, and the numbers of persons with no HIV infection. By the way, the false-positive rate is (1-the specificity). An experiment to find the specificity also gives the false positive rate and vice versa.

How should we design an experiment to discover this important data on specificity?

Firstly, since the underlying problem is largely one of deciding between bona fide antibodies and cross-reacting antibodies, we must include in our sample persons who are likely to have a large repertoire of antibodies to agents other than HIV. The more the merrier. Thus we must include persons who are sick and who have diseases similar to AIDS but do not have AIDS.

Secondly, we need a way of determining the presence or absence of HIV infection. Obviously, this can't be the antibody test itself because that's what we're trying to validate. When we measure specificity, we are trying to find out how often reactions occur in individuals who do NOT have HIV infection. Rather surprisingly, in the AIDS literature, the specificity of the HIV antibody tests has been evaluated by testing for reactions in healthy individuals such as blood donors. These persons are chosen as de factos for the absence of HIV infection. Under such circumstances, few if any positive reactions are found but this is not necessarily, as the HIV/AIDS experts claim, because the tests are highly specific. In fact, this is the wrong experiment for two reasons.

Firstly, healthy people do not have large number or variety of antibodies to react with the test. That goes with being healthy. That's why we put them in the Army and let them donate blood. There are simply not enough antibodies available to measure the propensity for unwanted reactions. Secondly, good health cannot be used as a de facto for the absence of HIV infection any more than good health can be used as a de facto for the absence of gall stones, kidney stones, pregnancy, hydatid cysts, deep vein thrombosis, cerebral aneurysms, pathogenic bacteria or coronary artery disease.

The practice widely adopted by HIV/AIDS experts of appraising HIV antibody tests by testing thousands of healthy blood donors also creates an enormous dilemma: If healthy people are regarded as a de facto gold standard for the absence of HIV infection, by what criteria can similarly healthy people or even the same individuals be regarded as infected at some future date?

But back to the problem of validation. We select our thousand people who are sick. And let's make sure we include some who have diseases similar to AIDS, a few healthy persons, and some cases of AIDS as well—you never know, we might be in for a big surprise. We might find some AIDS patients who are antibody positive in the absence of HIV infection. In fact, if you read Gallo's May 1984 Science papers that claims HIV was proven to be the cause of AIDS, HIV could be "isolated" in less than half the AIDS cases.

Most of the people selected will have lots of antibodies and this will give the test a fair run for its money. But hold on. If HIV causes AIDS, and some of our patients have AIDS-like diseases—even those who are healthy—how do we get past the sticky problem of knowing who is or is not infected with HIV? We don't want to include those who may actually be infected in our analysis because we want to evaluate the test when there is no HIV infection.

By now, some of you will have arrived at the correct solution to the problem of distinguishing who is and who is not infected. It's rather obvious scientifically speaking—you have to use HIV itself. You must divide your blood samples from each person in two: One sample to test for the antibody reactions and the other to try and isolate HIV. To know what the HIV antibody tests tell you about HIV infection, you compare the reactions with what you are trying to measure. The only way to distinguish between real reactions and cross-reactions is to use HIV isolation as an independent yardstick or gold standard.

What are the results of such an experiment? How many of an appropriately chosen 1,000 patients from whom HIV cannot be isolated have an antibody reaction? How many of the 1,000 from who HIV can be isolated have no reaction? What does the medical literature tell us about this? What does the data accompanying HIV antibody test kits say? I can't tell you because—bizarre as it may seem—17 years since the discovery of HIV and 15 years since the development of the HIV antibody tests, this experiment has not been done. We still don't know how many positive tests occur in the absence of HIV infection. It might be none or it might be all. Nobody knows. There is no proof of the specificity of the HIV antibody tests for HIV infection.

What if someone decided to do this experiment to prove the specificity of HIV tests? Is it feasible? That's hard to say because it depends on how much importance you place on the precision of defining HIV infection. Ultimately, this can only be defined by the isolation of a unique retrovirus. The word isolation comes from the Latin word "insulatus" meaning "made into an island". It refers to the act of separating an object from everything else that is not that object, like solitary confinement.

The rules of retrovirus isolation are now old. All the HIV experts should know them. They were developed in the several decades preceding the beginning of the AIDS era in 1981 and were thoroughly discussed at a meeting held at the Pasteur Institute in 1973 and attended by now leading HIV/AIDS researchers including Barre-Sinoussi and Chermann. These are a set of rules that credibly achieve the aim of separateness. The problem is that no claim of HIV isolation yet presented fulfils either the island concept or follows these rules. None of these claims even fulfils the initial and most basic of these rules, the requirement to obtain an electron micrograph of the material that is present at a sucrose density gradient of 1.16 gm/ml.

In fact, no claim of HIV isolation is isolation. All such claims are based on a set of phenomena ranging from so-called HIV proteins such as p24, to reverse transcriptase enzyme activity, "HIV particles," or “HIV PCR” that are detected in cultures of tissues of AIDS patients, none of which is even specific for retroviruses. And without isolation, who can say whether the proteins used in the HIV antibody tests are unique to HIV? As Philip Mortimer and his colleagues from the UK Public Health Laboratory Service noted: "Experience has shown that neither HIV culture nor tests for p24 antigen are of much value in diagnostic testing. They may be insensitive and/or non-specific"[5].

Yes, I know that we have all been shown pictures of something called HIV, but in the extensive retrovirology literature, retrovirus-like particles are commonplace. For a start, insects, reptiles, fish and tapeworms have them. Retroviruses are also found in the majority of healthy human placentas. And while it is true that electron microscopy reveals retroviral-like particles in 90% of enlarged lymph nodes from AIDS patients, the identical particles can also be found in 90% of enlarged lymph nodes from patients who do not have AIDS and who are not at risk for developing AIDS [6]. If the particles seen in lymph nodes from AIDS patients are HIV (as the AIDS experts assure us), what are the particles seen in the lymph nodes of patients who are not at risk for AIDS and what is their relationship to the plethora of other particles seen tissue cultures from AIDS patients?

Wait, some may ask, what about the PCR test (the polymerase chain reaction test or “viral load”)? For those who don't know, PCR is a new and very sensitive technique for finding genetic blueprints. But can PCR validate the antibody tests? No, I'm afraid it cannot.

To perform the PCR you need to begin with a piece of RNA or DNA that you can say for certain belongs to the HIV genome. To obtain the HIV genome, you first need to isolate an HIV particle. That's where the HIV genome comes from and that is the only way to know the RNA or DNA that actually belongs to the virus. Even the most charitable interpretation of the data available to date does not show that a unique retrovirus, HIV, has been isolated. Furthermore, even if one assumes that the process of selecting the RNA and DNA molecules (molecular probes) used in the PCR are from the HIV genome, there are still many problems with the use of the PCR to prove HIV infection.

At best, the PCR detects single genes and most often, only bits of genes. If your PCR finds two or three genetic fragments out of a possible dozen complete genes is this proof that you have all the genes? The whole genome? No it is not, and in fact HIV experts admit that the majority of HIV genomes studied are defective. This means they are incomplete and could never orchestrate the synthesis of a viral particle. Even if all genomes were complete, let's not imagine for a moment having the blue prints means you've built the house. Basic retrovirology long teaches us you can carry a whole retroviral genome around inside your cells all your life without ever making a viral particle. And in 1992, in

the only study of its type, French researchers found the HIV PCR non-reproducible and the agreement between the PCR and the HIV Western Blot was found to vary between 40-100% and was especially poor when fragments of more than one gene were sought [7]. In this study, there were several PCR negative/HIV positive as well as several PCR positive/HIV negative samples. In other words, the two tests don't match. As far as which test proves HIV infection, you pay your money and take your pick.

Finally, a specificity in excess of 99.9% sounds pretty convincing, but is it? What if you were found to have a positive HIV antibody test? What is your chance of being truly HIV infected, and not a false-positive?

To answer this, let's imagine a population of one million people where somehow, by authentic isolation studies, we know 1/1000 persons are HIV infected. Let's also assume that there is definite proof measured against a viral isolation gold standard that the HIV antibody tests are 99.9% specific for HIV infection. If the test is also 100% sensitive, it will detect all of the 1,000 infected people. However, 0.1% (1-specificity) of the 999,000 non-infected remainder will also be seropositive. That's another 999 people making a total of 1,999 positive tests, 1,000 who are infected and 999 who aren't. In this case, if you were randomly selected and found to be antibody positive there is only a 50/50 chance you are actually infected. The test will be wrong half the time. But for most of you, we can probably do better than this because most of you are arguably somewhat removed from the risk groups that dominate the statistics. If your odds are only 1/2000 of being infected, and if we drop the specificity of the test slightly to a mere 99.6%, a positive test will be wrong in 89% of cases, or in other words, almost all of the time.

Where does all this leave HIV/AIDS patients?

Firstly, the only evidence that HIV is the cause of AIDS is the perception by the AIDS experts of a correlation between antibody reactions and the presence of AIDS-defining diseases. However, for AIDS patients who have had antibody tests and have been diagnosed HIV infected solely on the basis of these tests, we can argue that there is no proof that even one such patient is infected with a virus called HIV. Secondly, in these cases, the tests provide no justification for the administration of potentially toxic drugs like AZT on the basis of a perceived anti-viral activity. Certainly the HIV antibody tests confirm that certain diseases are AIDS rather than just those diseases but this can be construed as an artefact of definition.

The only scientific conclusion we are permitted to make is that in some but not all well defined at-risk individuals, there is a correlation between antibody reactions, whatever their raison d'etre, and the propensity to develop and die from certain diseases. On the other hand, if you're HIV positive but not in a risk group and especially if you're healthy, any pronouncements on your likely outcome will be severely confounded by knowing you are positive, a situation we might describe as 20th century bone pointing*. And your health may suffer further from the use of medications administered in good faith to kill a virus you may not have. The failure to verify the antibody tests against the gold standard of virus isolation is a serious omission of scientific method. In the absence of such validation, these tests should not be used to diagnose HIV infection.

Addendum

In the entire AIDS literature there is only one study, that of Colonel Donald Burke and his colleagues [3] from the Walter Reed Army Institute, that is widely regarded as the definitive proof of the specificity of the HIV Western Blot.

Over an eighteen month period, Burke and his colleagues tested 1.2 million applicants for US military service. Burke's testing procedure was a progression through two ELISAs and two Western Blots. From these data, the HIV seroprevalence was found to be 1.48/1000. Burke then retrospectively investigated a highly selected sample of this population in which the HIV seroprevalence was 1/10th that of the 1.2 million. This group comprised 135,187 persons aged 17-18 years who resided in rural areas where the cumulative incidence of AIDS was low. Many would assume this group to be no different from healthy blood donors and would regard all HIV positives found among them as false positives, but Burke and his colleagues' premises were the opposite.

Assuming there were true positives among these healthy, rural American youth and wishing to evaluate the false positive rate and specificity of the Western Blot, Burke needed to define HIV infection. This was done by performing a panel of four more antibody tests on sera from the 15 out of 135,187 applicants who had already been found twice ELISA and twice Western Blot positive.

Two of the extra four tests were other Western Blots and two were similar tests. Any individual found positive in all four of the extra tests, thereby making a total of eight positive antibody tests, was deemed HIV infected. Those who failed any of the extra four tests were deemed non-HIV infected. Of the 15, one failed to react on all four tests and thus Burke conceded only one, not 15, false-positives. From these data, Burke calculated the specificity of the HIV Western Blot to be in excess of 99.9%.

There are many flaws in this study and they are outlined in reference 1. Here I wish to draw to your attention to the fact that an antibody test, even if repeated and found positive a thousand times, does not prove the presence of a viral infection.

* Bone pointing is a traditional, ritualistic punishment practiced by Australian aborigines. A bone is pointed at an individual as a method of retribution. That individual soon becomes sick and death within weeks or months is an invariable consequence.

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FAQ's

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References

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