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The fallibility of HIV western blot

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In 1984, Gallo and his colleagues described the growth in continuous culture of the virus now called human immunodeficiency virus (HIV).[1] The virus antigens were characterised by means of the technique known as western blot[2] and, since then, western blot has become central to HIV serodiagnosis. Most authorities believe that it is still the best way to confirm the presence of antibodies to the virus though others think it too expensive and its interpretation too uncertain for routine use. They prefer to apply a combination of less expensive assays, an approach that is much more economical and of especial benefit to diagnostic services in developing countries.

Western blotting consists of several steps. Viral proteins previously extracted from infected cell culture by detergent treatment and hydrolysis are separated electrophoretically in a polyacrylamide gel and transferred as discrete bands onto a sheet of nitrocellulose paper. The sheet is cut across the bands into narrow strips. Patients' and control sera are applied to individual strips so that antibodies, if present, can react with the protein bands. A positive result is shown by incubation with an enzyme conjugated to antibody against human immunoglobulin, and a substrate that becomes coloured in the presence of bound enzyme. The transferred proteins are identified by the position of the coloured bands on the positive control strip and then, with the other strips arranged in parallel, patients' antibodies to individual HIV proteins can be recognised. Interpretation of these reactions is often difficult, even for experienced personnel.

Since 1984, cheaper and less demanding methods for detecting HIV antibodies have been developed. The enzyme-linked immunosorbent assay (ELISA) originally described by Gallo's group has been commercialised; modified ELISAs, particle agglutination tests, and other simple and rapid tests have also been devised.[3] These methods have been widely used as HIV-screening tests, but none has been generally accepted as an alternative to western blot as a confirmatory test. Clinical and laboratory staff have become wedded to the apparent extra sophistication and sensitivity of the western blot so that, uniquely for diagnosis of this virus infection, it is said to be necessary to identify to which native proteins serological responses are being made. Meanwhile, the advantages of western blot have been eroded by improvements in the accuracy of other HIV assays, and the best ELISAs are now equally sensitive and more specific. These photometrically read assays also obviate subjective bias and the other difficulties that arise from the use of a western blot in routine diagnosis—variability in quality of strips, difficulty in interpreting reactions, frequent indeterminate results and high cost. The price of a manufactured western blot strip is more than fifteen times that of an HIV ELISA screening test, mostly because it is difficult to maintain good representation of key envelope proteins (gp41, gp120, and gp160) in each lot.

Interpretation of western blot patterns differs according to the observer and laboratory, and attempts to standardise western blotting by establishing interlaboratory guidelines for reading the strips have been met with only limited success. Manufacturers list their own criteria for interpreting western blots, as do the Centers for Disease Control (CDC)[4] and at least five other US bodies. The World Health Organisation, which set criteria in 1988, revised them in 1990.[5] Individual laboratories may add criteria of their own, and in a recent quality assessment exercise it was found that participating laboratories had developed eleven different sets of criteria to read western blots. Confusion of this sort must lead to errors.

To minimise confusion, epidemiologists at CDC have tried to persuade US laboratories to use the western blot kit licensed by the Food and Drug Administration (FDA) and apply uniform interpretative criteria. As a result, western blot specificity in their Model Performance Evaluation Program rose from 91·6% in 1988 to 97·8% in 1989.[6] However, a California study has suggested that western blot testing remains inconsistent between laboratories, that HIV-positive sera are often reported as indeterminate, and that the high cost of a western blot can act as a deterrent to confirmatory testing being done at all.[7] The reluctance of the FDA to license enzyme immunoassays that are based on recombinant and peptide antigens, and could be used as cheaper confirmatory tests, can only have contributed to these difficulties.[8] The new generation of HIV assays is both more specific and, as shown on specimens from seroconverting plasmapheresis donors, at least as sensitive as the western blot.[9] The present FDA position will be hard to sustain, especially since the high sensitivity of the western blot is achieved only when criteria are applied that lead to many indeterminate reports. In blood donor studies in the developed world, about 20% of sera referred to confirmatory laboratories give indeterminate western blot results, almost all of which are on presumed negative specimens.[10] In these countries the continued use of the western blot to test donors who are repeatedly reactive in a single ELISA screening test gives rise to unnecessary work and anxiety that could be avoided by the use of supplementary screening assays.

The shortcomings of the western blot test would have been identified sooner if it had been evaluated as a condition of licensing and release onto national markets. Instead, this

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test has been used as the "gold standard" for other assays and the possibility that it might itself be inaccurate has largely been ignored. When results of several screening tests have suggested that a western blot result is wrong, the fault has often been attributed not to the blot but to observer error. However, the person who reads the strip is an integral part of the process that leads to a western blot result, and so observer performance should not be discounted in this way. Their interpretation, correct or not, is part of the test. Before confidence can be placed in the diagnostic accuracy of the western blot, local assessments are needed in which individual observers read strips without collusion. Unexpected variability may then reveal itself and if so its cause must be ascertained.[11]

There is strong evidence that positive anti-HIV reactions can be confirmed by combinations of screening assays.[12,13] This approach can also discriminate serologically between HIV1 and HIV2 infections. It is expensive and often unsuccessful to try to do this by western blot.[14] In addition, confirmation of negative screening reactions (which in areas of high HIV prevalence may be important) can be achieved by the use of supplementary screening tests.

Western blot detection of HIV antibodies began as, and should have remained, a research tool. Because of its high cost, the continuing improvements in other assays, and the need for reliable results in areas where there are few staff trained to read difficult subjective tests, the western blot is gradually being superseded. The challenge, especially in the developing world, will be to ensure that the money saved by this rationalisation is used to provide more resources for HIV screening in blood banks and elsewhere.

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BOOKSHELF

A History of Human Helminthology

By David I. Grove. Wallingford, Oxon: CAB International. 1990. Pp 848. £55/$96.25. ISBN 0-851986897.

Most clinicians who treat a child with threadworms, or deal with yet another lorryload of trans-African explorers who have picked up schistosomiasis, will be ignorant of the vast array of observation and experimentation which is the basis of our modern understanding of parasitic worm infections. Grove's book, the result of 12 years' work, is an attempt to describe man's struggle to come to grips with the origins and transmission of helminth diseases.

The appearance and smell of the book are unappealing: the print is unattractive and there are no pictures of the helminths or their intricate life-cycles, only portraits of some of the famous investigators. But readers who are not put off by external impressions will find much to enjoy. The coverage is extremely thorough, starting with the nomenclature and classification of worms, followed by chapters on the origin and transmission of worms, the discovery and development of anthelmintics, and then individual conditions. For each pathogen there is a valuable synopsis of current knowledge: a description of the discovery of the adult worm, larval stages, intermediate hosts, recognition of the clinical features, development of diagnostic methods, search for effective treatment, understanding of the epidemiology, and evolution of preventive and control measures. These discussions are well referenced and there are tables that summarise major landmarks in our understanding of each organism.

To attract attention in Ancient Egypt, helminths had to be large enough to be visible to the naked eye and independently motile to prove that they were alive; the microscope was undoubtedly the greatest single technical advance in the history of helminthology. There are many interesting examples of human and self-experimentation: Mosler (*Ascaris*), Barlow (*Fasciolopsis buski*), and Leuckart (*Enterobius*) all tried to infect themselves or others—sometimes with uncomfortable results—in studies that advanced our knowledge but might now be regarded as unethical. Many rare and obscure human pathogens are mentioned. Indeed, this book is one of the most comprehensive sources of information on human helminthiasis that I have been able to find. Those who want to know about ā-ā-ā disease, *Watsonius watsoni* infection, and cheilospiruriasis will all find satisfaction. It is humbling to be reminded of the amount of argument, ingenuity, and experiment required to establish the pathogenicity and life-cycles of these diverse parasites. A particularly fascinating example is echinococcosis: hydatid cysts were mentioned in the Talmud but their true nature and relation to the tapeworm were not understood until the 18th and 19th centuries. The entertaining penultimate chapter, Miscellanea, discusses various imaginary worms: toothworms, eyeworms, earworms, nasal worms, urine worms, umbilical worms, and corpse worms. Strongly recommended to those interested in helminthology, tropical infectious diseases and geographical medicine, or medical history.

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