AIDS

Two British blood tests launched

THE first stage of the British government's evaluation of commercially available kits to screen blood for the AIDS (acquired immune deficiency syndrome) virus, HTLV-III (human T-cell lymphotropic virus), was completed last week. The Virus Reference Laboratory of the Public Health Service has tested five manufacturers' kits (Abbott Laboratories Ltd, Electronucleonics Ltd, Organon Teknika Ltd, Ortho Diagnostic Systems and Wellcome Diagnostics). Three performed appreciably better than the others in distinguishing clearly between negative, positive, false-positive and heat-treated sera. Two of these, from Wellcome (Britain) and Organon (the Netherlands), were found to be especially easy to use and are now to be tested by the Blood Transfusion Service on about 6,000 samples. When this evaluation is complete, routine screening of blood donations will be introduced, probably by mid-September.

Screening will protect those most vulnerable to the AIDS virus, that is, people dependent on blood transfusions. For the present, "at risk" individuals have been asked not to donate blood. But AIDS is unlikely to stay contained within some sections of the population. Already there are reports of the presence of antibodies to the virus in women in some African cities, confirming that the disease can be transmitted by heterosexual contact.

Although the kits will greatly increase the efficiency of detecting the AIDS virus in sera, and hence containing its spread, a cure for the disease is no closer, although recent results indicate that the drug Imunovir can help patients with AIDS-related complex or lymphadenopathy syndrome.

The AIDS virus replicates within the body's immune system and, although it is highly infectious, only 3 per cent of people infected with HTLV-III develop AIDS; however, another 20 per cent develop illnesses of varying severity ranging up to persistent generalized lymphadenopathy.

AIDS is almost always fatal, but the virus has a long incubation time. In July 1985, about 10,000 people in the United Kingdom and a million in the United States were estimated to be infected with HTLV-III. Until treatment is available, the first step in management of the condition is to prevent the spread of infection by screening blood for HTLV-III antibodies. The kits evaluated last week accurately identify the presence of HTLV-III antibodies in the blood. The next development will be to develop a test for the antigen, which will allow earlier identification of the virus, before an individual has made antibodies. It is hoped that antigen tests will be easier to perform and more sensitive than antibody binding.

Of the tests chosen for further evaluation in Britain, Wellcome's is a competitive enzyme-linked immunosorbent assay (ELISA) and Organon's, in common with the unsuccessful tests, is an indirect ELISA. The Wellcome kit consists of a plastic well first coated with "control" HTLV-III antibody and then with impure HTLV-III antigen. (This procedure purifies the antigen.) The user adds to the well a test sample of serum together with a conjugate of control antibody linked to the enzyme horseradish peroxidase. HTLV-III antibodies in the sample compete with the enzyme-linked antibody in binding to the antigen. Unbound components of the mixture are washed out, and the substrate of the enzyme added to the wells. Any enzyme still bound to the wells is seen as a blue colour, and when the reaction is stopped with sulphuric acid, a yellow colour develops. If antibodies were present in the sample, some will have bound to the antigen; because they are not enzyme-linked, less colour develops.

The indirect ELISA also starts with antibody–antigen-coated wells. The sample is then added, washed, then enzyme-linked human anti-immunoglobulin G (IgG) is added to the mixture. Any antibody in the sample will bind to the anti-IgG and the development of colour indicates HTLV-III antibodies are present.

In both tests, samples scoring positive are subjected to a confirmatory test, such as Western blotting, in which the constituent proteins of the virus are separated on a two-dimensional gel and identified by their relative molecular mass.

The Wellcome test may have an advantage over the indirect ELISA because there are fewer steps (five against Organon's eight and taking two hours rather than just under three), there is not as much sample dilution and it is potentially more specific as it uses the principle of competition rather than binding to a more general antibody. However, the National Institute of Biological Standards and Control has found no difference in specificity between the two successful tests on a limited number of samples. **Maxine Clarke**

AIDS

US blood-bank tests established

Washington

SECOND-generation tests for the AIDS (acquired immune deficiency syndrome) virus that may reduce the persistent problem of false positives have produced encouraging early results. Abbott Laboratories, Centocor and Travenol–Genentech are among US companies evaluating enzyme-linked immunosorbent assay (ELISA) tests that incorporate recombinant antigen, which avoids at least some of the problems caused by the natural variability of the virus.

Some preliminary data on the new tests were given last week at a government-sponsored conference at the National Institutes of Health. In addition, Abbott claims to have a prototype test that detects viral antigens in the blood, as distinct from the human antibodies that existing ELISA tests look for. A workable antigen test would be a much surer guide to those at risk of developing the disease or to carriers of the disease; it is, however, technically more demanding because antigens are present in much lower concentrations than antibodies.

The existing licensed tests on the UK market, supplied by Abbott, Electronucleonics and Litton, are generally agreed to have effectively protected blood banks; virtually all blood samples from AIDS patients are detected. But there remains a substantial number of repeatedly reactive samples that do not show evidence of antibodies on a Western blot test, even when technical errors are excluded.

In Western blotting, the principle of the better-known Southern and Northern blotting techniques is extended to the detection of proteins rather than nucleic acid fragments. The protein mixture for assay is size fractionated by gel electrophoresis, followed by transfer to a support in such a way as to preserve the relative positions of the separated proteins. Specific proteins attached to the support can then be detected with antibodies.

The Western blot is considered more reliable than ELISA testing, although even so it shows appreciable variation between laboratories, according to Carl Saxinger of the National Cancer Institute. One reason for the large number of false positives is mutation in the virus populations that supply the antigens used in tests. Recombinant antigens would be more stable (although the problem of sample virus variability would remain) and so perhaps reduce the false-positive rate. A cocktail of different recombinant antigens might be the best solution. Recombinant antigens have the additional advantage of avoiding the need to culture live virus.

The false-positive problem has led to harrowing decisions about what to tell patients whose samples appear positive, although manufacturers stress that current tests are not intended for use in diagnosis. The presence of antibodies, even if confirmed, does not necessarily imply that overt disease will develop. Looking to the future, some manufacturers expect to be producing batteries of confirmatory tests for antibodies characteristic of different stages of infection and disease. Together with a viral antigen test, these may allow immune people to be recognized and thus provide useful data on the progress of infection. **Tim Beardsley**