DIAGNOSTICS

EUROPE GETTING INTO THE HIV-TESTING ACT

LONDON—Compared with the large number of U.S. companies in the highly competitive business of blood-screening tests for human immunodeficiency virus (HIV) (see *Biol Technology* 6:259–264, Mar. '88), European firms are still few. In technical terms, however, they seem to be holding their own, especially when it comes to tests for HIV-2.

Among the first batch of enzyme-linked immunosorbent assays (ELISAs) that detect HIV antibodies, the only European test was also the only one to be based on the principle of competitive inhibition. Moreover, it alone employed a microwell surface that was not directly coated with antigens from HIV lysates, but first coated with HIV antibodies, which in effect then immunopurify the lysate antigens onto the surface. The resulting test has been widely adopted for U.K. blood-donor screening because it gives fewer false positives than its rivals, maintains manufacturer Wellcome Diagnostics (Dartford, U.K.).

In a new version of the test, just launched, a mouse monoclonal antibody to HIV replaces the polyclonal antibody used to coat the microwells. Wellcome claims that this switch increases sensitivity and decreases variability, while maintaining specificity. Other European companies have stuck to the direct coating of the ELISA solid phase with antigens, even when, like Behringwerke (Marburg, F.R.G.), they have taken to competitive inhibition formats.

A variant on the more usual theme is the Karpas AIDS Cell Test, invented by Abraham Karpas of Cambridge University. Sold by Fuji Chemical Industries (Toyama, Japan) and Italfarmaco (Milan, Italy), the test's novelty lies in its use of acetone-fixed HIV-infected T cells in place of purified antigens. For detection, the test uses a protein A-peroxidase conjugate; equivocal results can usually be resolved by microscopic inspection of the cytological distribution of stain. Karpas says that this inexpensive and simple test can be used for both screening and confirmation, and it works for both HIV-1 and HIV-2.

HIV-2 Testing

Diagnostics Pasteur (Marnes-la-Coquette, France) has the first HIV-2 antibody tests on the market, thanks to its links with the Pasteur Institute where the virus was first isolated. (The Institute and the company have recently filed a complaint against DuPont, Wilmington, DE, for infringe-

ment of their patents on HIV-2 test kits.) Although very few cases of HIV-2 infection are yet found outside Africa, some blood transfusion centers have begun to screen for HIV-2 antibody, particularly if there is an African connection. Unlike the first Diagnostics Pasteur tests, however, the latest HIV-1 and HIV-2 tests include a control for non-specific reacting antigens in the viral lysate.

In another effort, Clonatec (Paris) hopes soon to commercialize its synthetic peptide-coated microwell test for HIV-2 antibodies, which the company claims is far more specific than antigen-based tests.

Second-Generation ELISAs

Recombinant DNA antigens or synthetic peptides can replace viral antigens derived from cell lysates to create so-called second-generation ELISA tests. These are expected to have increased specificity and to be able to detect antibodies against particular HIV antigens, if desired.

Biochrom (Berlin, F.R.G.) has the first European test based on synthetic peptides and claims that the assay produces no false positives and fewer false negatives than first-generation tests. In one format, the test uses a combination of four peptides to detect HIV antibodies; in another it tests separately for the presence of antibodies against p15, p18, p24 (core), gp41 (transmembrane), and gp120 (envelope) proteins of HIV-1.

Organon Teknika (Turnhout, Belgium) is well advanced with specific tests for gp41/120 and p24. The former uses a recombinant antigen in a competitive inhibition ELISA; the lat-

ter is a sandwich ELISA using two different monoclonal antibodies. Both will be one-step tests on undiluted serum, and the company's product line manager, Paul Geenen, claims that they are at least as sensitive as Western blots.

Antigen Tests

Two European companies have so far produced tests for HIV-1 antigens, which can often be detected before antibodies. In some patients antigens fade away as antibodies increase; their reappearance is a poor prognostic sign.

Both companies base their antigen test on sandwich ELISA technology, in which serum antigen is captured by solid-phase antibody and then detected with soluble antibody. Organon Teknika links its second antibody to horse radish peroxidase for detection purposes, whereas Diagnostics Pasteur uses avidin-biotin.

Future Tests

Among interesting antibody tests undergoing development in Europe are a three-minute whole blood test and a saliva test. The blood test is a collaborative French-U.S. project involving Clonatec, V-Tech (Pomona, CA), and Syntro (San Diego, CA). The test uses an immunofiltration device, containing a synthetic-peptide coated membrane. After a blood sample is filtered through the membrane it is followed by an enzyme-linked anti-immunoglobulin and substrate. With different peptides on different regions of the membrane, at least four different antibodies can be detected simultaneously. Clonatec's research director, Philippe Pouletty, says that independent tests have confirmed that this three-minute test can be 100 percent specific for HIV-1 if the right peptide is used.

The saliva test is being developed at the U.K. government's Central Public Health Laboratory (London), where many commercial tests have been evaluated (although official approval is not necessary for the U.K. sale of diagnostics). It is an antibody capture assay in which the captor is immunoglobulin G (or A) coated on polystyrene beads. Ten-fold diluted (but otherwise untreated) saliva is incubated with the beads, after which they are further incubated first with HIV antigen and then with radioisotope- or peroxidase-labeled HIV antibody. Results published in *Lancet* a year ago showed little difference between the two labels.

—Peter Newmark