Enzyme Immunoassay for HTLV-III Antibody

Method

The Abbott Laboratories Enzyme Immunoassay for antibody to Human T-cell Lymphotrophic Virus (HTLV III EIA) was approved for clinical use in March 1985, and similar assays were undergoing clinical trials at that time. The Abbott Kit (1) uses beads coated with antigen from disrupted, detergent-inactivated HTLV-III virus. A 1:20 dilution of plasma or serum is incubated with a bead for one hour at 40 degrees C. The bead is washed, and re-incubated with a conjugate of goat anti-human IgG and horseradish peroxidase. The bead is washed again, and substrate solution containing ortho-phenylenediamine (OPD) and hydrogen peroxide is added. If the patient specimen contained antibody to HTLV-III, peroxidase on the bead will catalyze the transfer of electrons from hydrogen peroxide to OPD to produce a colored product. The reaction is stopped with sulfuric acid after 30 minutes, and any resulting yellow-orange color is read at 492 nm (A-492). To calculate the cutoff absorbence value, the mean absorbence of the negative controls is added to one tenth of the mean absorbence of the positive controls (two negative and three positive controls are assayed with each run). Specimens with A-492 values less than the cutoff value are negative, and those with A-492 values greater than or equal to the cutoff value are considered reactive. Abbott recommends retesting all reactive specimens, and interpreting a specimen as positive only if it is repeatedly reactive.

Technical and Clinical Limitations

The assay procedure requires approximately four hours and attention to numerous details, and thus has many potential sources of error. The calculation of results includes several checks to determine when a run is flawed and must be repeated. The peroxidase reaction may be a particularly trouble-prone step since the OPD substrate solution is unstable and must be used within 60 minutes of preparation. Overall, the method probably includes sufficient checks to detect major systemic errors.

The HTLV III EIA is designed "to screen blood and plasma donations so that units containing antibody can be identified and eliminated" and "is inappropriate to use as a screen for AIDS or as a screen for members of groups at increased risk for AIDS in the general population." (1) The most desirable feature of a screening test is high sensitivity; the specificity becomes important when the cost of false-positives becomes significant. Abbott estimates the sensitivity and specificity to be 93.4% and 99.8%, respectively, based on assumed 100% prevalence of HTLV III antibody in AIDS patients and
zero prevalence in random donors. Since all AIDS patients may not have detectable antibodies (2-4), the actual sensitivity is probably higher. A similar EIA showed sensitivity and specificity of 97.3% and 98.6%, respectively (3). The probable cost and impact of testing on the United States blood supply can be calculated from published estimates of HTLV III antibody prevalence. Abbott tested 9,949 serum and plasma samples from blood donors and found 0.22% to be repeatably positive (corresponding to a prevalence of 0.252%, or 252 per 100,000). With a false positive rate of 0.2%, rejecting a unit on the basis of a single test would cause 0.434% of blood to be discarded, or one in every 230 units. It has been estimated that testing the 12 million units of blood collected yearly in the United States would cost $60-120 million (6), and could prevent most cases of transfusion-associated AIDS. (Transfusion-associated AIDS comprises about 2% of all cases (4); up to 40,000 patients are expected to develop AIDS during the next two years (5); so about 400 transfusion-associated cases may develop yearly.) Assuming HTLV III antibody in all potentially infective units and 93.4% test sensitivity, 373 cases would be prevented at a cost of up to $321,000 per case. Transfusion-associated AIDS, however, is expected to continue rising as previously infected patients develop the disease, and the decrease may not be seen for several months or more.

Clinical Applications

The data obtained by Abbott and others suggest that HTLV III antibody testing will reduce the number of antibody-containing units in the banked blood supply. The diagnostic value of the test in an individual patient is uncertain, however. A positive HTLV III EIA indicates antibody production but does not assess the presence of potentially contagious virus. Seropositivity may indicate that the patient is infected with HTLV III and will contract AIDS, or may have developed effective immunity (7). Limited studies have shown a 4.0-6.9% yearly rate of AIDS development in seropositive persons (5,8), but longer periods of evaluation and larger patient populations are required to determine the actual rate of disease development. The predictive value of the HTLV III EIA varies with prevalence, but tends to be low. The estimated prevalence of HTLV III antibodies in various blood donor populations ranges from 0.057% to 0.52%, which yields a positive predictive value range of 21-71% (1). Considerable controversy exists about informing the donor, but the consensus seems to be that a single positive test is insufficient evidence of presumptive infection (1,4). The presence of antibody should be confirmed with a second EIA or an alternate test, such as the Western blot, before informing the donor. The use of a second test will eliminate almost all of the false positives and produce a very high positive predictive value (over 99% using Abbott's data). The issue of notification is clouded by the problem of increased numbers of high risk donors donating blood to assess their antibody status; and a strong case can be made for withholding notification until the test becomes widely available. With some modifications, this test might be useful for screening patients instead of donated blood units, and may assume a diagnostic role as the relationship of HTLV III infection to AIDS becomes better understood.


