Titration of Human Serum Antibodies to *Toxoplasma gondii* with a Simple Fluorometric Assay

K. W. WALLS* AND E. R. BARNHART

Parasitology Division, Center for Disease Control, Public Health Service, U.S. Department of Health, Education and Welfare, Atlanta, Georgia 30333

Received for publication 2 September 1977

A new technique, FIAX, has been evaluated for the serodiagnosis of toxoplasmosis. It is based on a "dipstick" principle, and a special fluorometer is used to perform the indirect immunofluorescence test. The procedure appears to be simple and rapid and merits consideration as a useful serological test for toxoplasmosis.

Toussaint and Anderson (3) and Toussaint (2) have described the soluble-antigen fluorescent-antibody (SAFA) technique for serodiagnosis. Later, Sadun and Gore (1) compared the SAFA technique with the immunofluorescent-antibody test for schistosomiasis and found the SAFA technique to be less sensitive. Problems involving fluorometry interfered with the sensitivity and reliability of the SAFA test. Recently, a simple, accurate, and sensitive fluorometer has been developed for the determination of antinuclear antibody (ANA) and globulin levels in human sera (FIAX System, IDT, Santa Clara, Calif.) This system utilizes a "dipstick" principle in which antigen is adsorbed to a cellulose acetate disk affixed to a plastic handle (FIAX, StiQ Sampler). The disk is placed in diluted patient's serum, washed, transferred to conjugated anti-globulin, washed, and inserted into the viewing stage of the special fluorometer, where a computerized quantitative result is read and printed.

Because of the apparent simplicity, speed, objectivity, and economy of this system, we evaluated it with the soluble toxoplasma antigen. Initial results indicate that the system is practical, simple, and quantitative over a relatively broad titer range and that a single serum dilution is sufficient to determine the antibody level.

A modification of the FIAX protocol for ANA has been devised. Disks were coated with 10 μ l of an optimal concentration (1.65 mg of protein per ml) of a pressure-disrupted *Toxoplasma gondii* antigen; they were then allowed to air dry and were stored at 4°C until used.

In the test, disks were shaken for 30 min at room temperature in 0.5 ml of 1:51 patient's serum. They were then shaken for 5 min at room temperature in 0.5 ml of 0.1% Tween-20 (Atlas Chemical Co.) in 0.05 M tris(hydroxymethyl)-aminomethane-buffered saline (pH 8) wash. The disks were then transferred to 0.5 ml of an

optimal dilution of fluorescein isothiocyanate-labeled goat anti-human immunoglobulin G and shaken for 30 min at room temperature. After a second 5-min wash as described before, the disks were inserted in the fluorometer for a quantitative reading. Results are expressed as "fluorometric signal units" (FSU) as displayed by the fluorometer.

Figure 1 shows the curves resulting from plotting FSU for each dilution of a group of sera representing a broad range of indirect immunofluorescence (IIF) titers. Every serum, even that with a 1:16 titer, showed a prozone-type phenomenon at the lower dilutions. However, each strength of serum has a distinctly different curve. At the 1:40 to 1:80 dilutions, the separation of signals was maximal.

Because of this and because of the ease of preparing a 1:51 dilution (10 μ l in 0.5 ml), sera representing a range of IIF titers were tested at this dilution only. Figure 2 shows IIF titers plotted against the corresponding FSU. Partly because of the problems due to subjective measuring of IIF titers, FSU readings overlap at each IIF level, but a distinct difference in mean FSU

FIG. 1. FSU at each dilution of five sera representing a wide range of reactivity.

FIG. 2. Correlation of FSU with IIF titers for 50 sera. SD, Standard deviation.

values is seen between different IIF titer estimates. Obviously, there is a direct correlation between FSU at 1:51 and IIF titers. A plot of the mean FSU for each titer category would show a nearly straight-line relationship.

These data indicate that the FIAX system is a rapid, simple diagnostic test which gives a quantitative measure with a single reading. Firm parameters for performance of the test must be established, and large batteries of serum samples must be examined to determine the exact sensitivity and specificity of the test.

LITERATURE CITED

1. Sadun, E. H., and R. W. Gore. 1967. Relative sensitivity of soluble antigens (metabolic and somatic) and whole cercariae in fluorescent antibody tests for schistosomiasis in humans and rabbits. *Exp. Parasitol.* **20**:131-137.
2. Toussaint, A. J. 1966. Improvement of the soluble antigen fluorescent-antibody procedure. *Exp. Parasitol.* **19**:71-76.
3. Toussaint, A. J., and R. I. Anderson. 1965. Soluble antigen fluorescent-antibody technique. *Appl. Microbiol.* **13**:552-558.