Evaluation of MUREX SUDS Toxo Test

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The SUDS Toxo test (MUREX Corp., Norcross, Ga.) was compared with the indirect hemagglutination test (IHA) and the indirect fluorescent-antibody test (IFA) by examining 404 serum specimens, including 64 (15.8%) specimens with IFA titers of $\geq 1:2$. When SUDS was compared with IHA, sensitivity (96.4%), specificity (97.9%), and negative predictive value (99.4%) indicated that there were similar reactivities between the two tests. When an IFA titer of $\geq 1:16$ was considered significant and IHA and SUDS were compared with IFA, IHA was slightly less sensitive but had a higher positive predictive value than did SUDS; however, there was no statistical difference between the tests. When SUDS was compared with IFA, in which a titer of $\geq 1:16$ was considered significant, the high negative predictive value (100%), excellent sensitivity (100%) and specificity (98.3%), and ease of performance made SUDS an attractive alternative to IHA for screening single serum specimens for toxoplasmosis.

Toxoplasmosis, a disease with a worldwide distribution, is caused by the coccidian parasite *Toxoplasma gondii*. Previous serological surveys of populations in the United States have placed the number of antibody-positive individuals at somewhere between 3 and 40%, depending on age and geographical area (5, 6). The disease is particularly problematic when acquired during pregnancy since there is significant risk of congenital infection (4). A resurgence of interest in the disease has occurred because it is one of a number of opportunistic infections that occurs in immunocompromised hosts.

Primary infection with *T. gondii* is accompanied by production of immunoglobulin M (IgM) antibody within the first week following exposure. IgM antibody reaches a peak titer in 2 to 4 weeks and gradually declines to an undetectable level in 3 to 4 months. IgG antibody titers are detectable within 2 weeks after infection and reach peak titers between 2 and 6 months after infection. IgG antibody remains detectable for life. Because immunocompetent individuals may demonstrate high antibody titers to *T. gondii* in the absence of clinical symptoms, serological tests are the primary means of achieving a diagnosis of toxoplasmosis.

The Sabin-Feldman dye test, indirect hemagglutination test (IHA), and the indirect fluorescent-antibody test (IFA) are the best characterized and most frequently used serological tests (8, 14, 21). Excellent correlation exists between the Sabin-Feldman dye test and IFA, and IFA offers the advantage of detecting both IgM and IgG antibody (21). IHA is a useful screening test but suffers from nonspecific reactivity because of a heterophile antigen (7) and because it is insensitive in early and congenital toxoplasmosis (3).

The enzyme-linked immunosorbent assay (ELISA) for toxoplasmosis was first described by Voller et al. (19). Walls et al. (20) modified the test by substituting soluble antigen and adapted it for performance in a semiautomated microtiter system. Several investigators subsequently reported the use of ELISA systems for the evaluation of IgG and IgM response to toxoplasmosis (1, 10, 11, 13, 16, 17, 22). ELISA systems have been compared with the Sabin-Feldman dye test, IFA, IHA, and the complement fixation test and results

have been found to compare most closely with those of the complement fixation test and IFA (2, 18, 23).

Several diagnostic kits for the serodiagnosis of toxoplasmosis are commercially available. One of the most recent and novel kits to be released and the one evaluated in this study is the SUDS Toxo test (MUREX Corp., Norcross, Ga.). The test is a rapid, qualitative, colorimetric immunoassay for the detection of specific antitoxoplasma antibody in a serum specimen. The test requires 50 μ l of serum and provides results in less than 10 min. The procedure employs latex particles coated with soluble toxoplasma antigen. A serum specimen is reacted with the latex-antigen suspension, and a diluent is added and poured into the disposable SUDS test cartridge, which is designed to retain the latex and allow rapid, efficient washing of unreacted components. Anti-human IgG conjugated to alkaline phosphatase is added to the cartridge, in which it reacts with any anti-toxoplasma antibody complex bound to the latex particles. Following another wash, a chromogenic substrate is added. If the specimen contains toxoplasma antibodies, a blue color is produced which is detected visually in the bottom viewing area of the SUDS test cartridge. The test is intended as a rapid screening test for determining immune status with a single serum specimen.

The present study was undertaken to determine the performance of SUDS compared with that of IHA by using 404 serum specimens with various degrees of reactivity as defined by IFA. We also evaluated an experimental reader produced by the MUREX Corp. which was designed to print absorbance values that were spectrophotometrically determined from the reactivity visible in the opening in the bottom of the SUDS test cartridges. Results are presented for both visual and spectrophotometric readings of SUDS compared with those of IHA and IFA.

MATERIALS AND METHODS

Sera. Whole clotted blood or serum specimens for the diagnosis of toxoplasmosis were referred to the Hygienic Laboratory, University of Iowa, from physicians throughout Iowa. Serum samples were tested as they were received or stored at 4°C until they could be included in a test panel,

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which was performed at least once or sometimes twice each week. Specimens containing particulate matter or microbial contamination were clarified by centrifugation prior to testing. Unheated serum samples were used in all tests. Serum samples with any degree of reactivity in any of the test systems evaluated were stored at -70°C after testing to allow follow-up testing to resolve any discrepant results between tests. A total of 404 serum samples were included in the study. Of these, 64 (15.8%) were reactive with a titer of $\geq 1:2$ by IFA.

IFA. The quantitative IFA was performed as described by Kagan (9), except that dilutions of 1:16, 1:64, 1:256, and 1:1,024 were included in the initial examination of each serum specimen. Serum samples from patients with possible early and ophthalmic toxoplasmosis were tested at dilutions of 1:2, 1:4, 1:8, and 1:16, and IgM titers were determined on serum samples from patients with congenital toxoplasmosis and on physician request. IgM titers of 1:2 in infants and adults were considered significant. Reagents used for IFA were obtained from the Microbiological Research Corp., Bountiful, Utah.

IHA. The qualitative IHA for determination of antibodies to *T. gondii* in serum was performed by using the TPM-Test, which is commercially available from Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N. J. The procedure was performed exactly as described in the instructions that were provided with the test kit. Each serum specimen was tested at a single dilution of 1:64. Hemagglutination patterns between 1+ and 4+ were recorded as a titer of $\geq 1:64$. Equivocal test results were recorded as $\pm 1:64$, and negative test results were recorded as $< 1:64$. All specimens which tested $\pm 1:64$ or $\geq 1:64$ were quantitatively tested by IFA, and these serum specimens were considered IHA reactive for antibody to *T. gondii* for comparison with IFA and SUDS.

SUDS. SUDS was performed by using the instructions provided with the test kit. All reagents were stored at 4°C except for the wash and diluent, which were stored at room temperature (17 to 25°C). The SUDS test cartridges and related accessories were stored at room temperature. To perform the assay, up to 10 SUDS test cartridges (8 patient serum samples plus positive and negative controls) were placed on the laboratory bench and identified separately. SUDS sample cups were placed into the cartridge, and one drop of patient serum or control serum was added to each cup with a disposable micropipette. One drop of latex-antigen suspension was added to the SUDS sample cup, gently mixed with the serum sample, and allowed to incubate for 2 min. Next, 1 ml of diluent was added from a calibrated dropper supplied with the kit. The contents of the SUDS sample cup were poured into the SUDS test cartridge, and the SUDS sample cup was discarded. After the cartridge funnel emptied, 1.5 ml of wash reagent was added. When the funnel was again empty, one drop of enzyme-antibody conjugate was added, and the reaction mixture was allowed to incubate for 2 min. Next, 1.5 ml of wash reagent was added to the cartridge, and the funnel was allowed to drain. One drop of substrate was added, followed after 2 min by four drops of enzyme-substrate inhibitor. Color development stopped, and the cartridges were turned over to observe test reactivity in the center circle on the bottom of each cartridge. A distinct blue was considered a positive test when the center circle was compared with the two outer circles, which remained white and served as negative controls. Equal color development in all three circles was an indication of insufficient washing. Both positive and negative

TABLE 1. Results of IHA compared with those of IFA^a

IFA	No. of samples with the following IHA results ^b :	
	Reactive	Nonreactive
Reactive	53	2
Nonreactive	2	332

^a A titer of $\geq 1:16$ was considered reactive.

^b Sensitivity, 96.4%; specificity, 99.4%; efficiency, 99.0%; positive predictive value, 96.4%; negative predictive value, 99.4%.

control sera were included in the kit for comparison with test sera. Any test result which was inconclusive was repeated. The kit instructions included a printed color guide to assist with interpretation of the test results.

Experimental reader. The experimental reader was a microprocessor-controlled recording spectrophotometer designed to read the color intensity appearing in the center circle on the bottom of the SUDS test cartridge. After the visual readings were manually recorded, the reader was calibrated with an unreacted SUDS test cartridge at 570 nm. The negative control cartridge was read at 610 nm, and the offset was calculated automatically. A_{610} values were determined for the positive control and all serum specimens being tested. The resulting A_{610} values were used in the comparison of the reactivity of serum samples by IHA and IFA.

RESULTS

Of the 404 specimens included in this study, 14.1% ($n = 57$) demonstrated IFA titers of $\geq 1:16$, and 15.8% ($n = 64$) demonstrated IFA titers of $\geq 1:2$. The IFA titers and the number of serum samples at each titer were 1:2 ($n = 6$), 1:4 ($n = 1$), 1:16 ($n = 33$), 1:64 ($n = 13$), 1:256 ($n = 4$), 1:512 ($n = 2$), 1:1,024 ($n = 3$), and 1:2,048 ($n = 2$). Specimens containing an insufficient quantity of serum for all tests or specimens for which IgM antibody titers were specifically requested were tested initially by IFA; thus, IHA was not performed on 15 specimens. When 389 of 404 serum samples were tested by IHA, 13.1% ($n = 51$) were found to be reactive at a titer of $\geq 1:64$, and 14.1% ($n = 55$) were reactive at a titer of $\geq 1:64$ or $\pm 1:64$. Specimens which tested $< 1:64$ by IHA and which were nonreactive in SUDS were not tested by IFA. These specimens were considered nonreactive in comparisons with other tests. All 404 specimens were tested by SUDS, and 15.6% ($n = 63$) were determined to be reactive.

Data for comparison of the IHA and IFA results, in which an IFA titer of $\geq 1:16$ was considered significant and any IHA activity equal to or greater than $\pm 1:64$ was recorded reactive, are presented in Table 1. The high test efficiency (99%) and negative predictive value (99.4%) of IHA in this study provided a rigorous benchmark on which to evaluate SUDS.

A comparison of IHA results with SUDS results based on visual readings is presented in Table 2. Compared with IHA, SUDS had excellent sensitivity (96.4%), specificity (97.9%), and efficiency (97.7%). The positive predictive value (88.3%) and negative predictive value (99.4%) of SUDS were very good for a screening test. When results of discrepant serum samples were analyzed, the two IHA-reactive, SUDS-nonreactive samples had IFA titers of $< 1:2$ and $< 1:16$, thus suggesting that the reactive IHAs were false positive. Of the IHA-nonreactive but SUDS-reactive serum samples, two demonstrated IFA titers of 1:16, and the remaining five samples had IFA titers of 1:2 or 1:4.

TABLE 2. Results of SUDS compared with those of IHA

IHA	No. of samples with the following SUDS results ^a :	
	Reactive	Nonreactive
Reactive	53	2 ^b
Nonreactive	7 ^c	327

^a Sensitivity, 96.4%; specificity, 97.9%; efficiency, 97.7%; positive predictive value, 88.3%; negative predictive value, 99.4%.

^b Both serum samples tested <1:16 by IFA (one sample, <1:2; one sample, <1:16).

^c All serum samples tested ≥1:2 by IFA (four samples, 1:2; one sample, 1:4; two samples, 1:16).

Because SUDS correlated better with IFA than with IHA, we compared visual and spectrophotometric test results with IFA results, in which significance was defined as a titer of ≥1:16 and ≥1:2 (Tables 3 and 4). When an IFA titer of ≥1:16 was considered significant and SUDS was read visually, no false-negative results and six false-positive results were recorded; however, all six samples had IFA titers of 1:2 ($n = 5$) or 1:4 ($n = 1$). When an IFA titer of ≥1:2 was considered significant and SUDS was read visually, there were no false-positive results and one false-negative result on a serum sample with an IFA titer of 1:2. There was no significant difference in sensitivity, specificity, efficiency, or predictive value between visually read SUDS and IFA, whether significance was defined at a titer of ≥1:16 or ≥1:2. The positive predictive value was slightly lower with a visual reading and an IFA cutoff of ≥1:16.

By using spectrophotometric readings with a cutoff of ≥0.100 A_{610} units as a positive test, SUDS produced four false-positive and one false-negative result when compared with IFA, in which a titer of ≥1:16 was considered reactive. The false-negative SUDS had an A_{610} value of 0.055 units, yet it was weakly positive when read visually. The four false-positive SUDS results determined by using spectrophotometric readings and an IFA cutoff of ≥1:16 had IFA titers of 1:2 ($n = 3$) or 1:4 ($n = 1$), and all four samples were visually positive by SUDS. The one false-negative SUDS result had an IFA titer of 1:16 and was positive when read visually.

When spectrophotometric readings were used with an IFA cutoff of ≥1:2, no false-positive and four false-negative SUDS results were recorded. Visual SUDS results were positive for three of four of these samples, and only one sample had an A_{610} of <0.050 units. When all other tests were nonreactive, all visually negative samples with IFA titers of <1:2 or <1:16 had A_{610} values of ≤0.046 units. There was no significant difference in sensitivity, specificity, efficiency, or predictive value between visual and spectrophotometric readings of SUDS compared with those of IFA, with a significant titer defined as ≥1:16 or ≥1:2. A summary of these data is presented in Table 5.

The IHA results were plotted against A_{610} values obtained by SUDS (Fig. 1). All except four samples which tested <64 by IHA had A_{610} values of <0.100. The four false-negative IHA samples had A_{610} values between 0.144 and 0.226 and IFA titers of 1:2 ($n = 2$), 1:4 ($n = 1$), and 1:16 ($n = 1$). Of the four samples which had a titer of ±1:64 by IHA, two were clearly nonreactive and two were clearly reactive when tested by the IFA and SUDS. Figure 2 presents the distribution of IFA titers by A_{610} values obtained by using SUDS with spectrophotometric readings. Of 57 serum samples with IFA titers of ≥1:16, 54 had an A_{610} value of ≥0.168, and 61 of 64 serum samples with IFA titers of ≥1:2 had A_{610} values

TABLE 3. Results of SUDS compared with those of IFA^a

Test	No. of samples with the following SUDS results:			
	Visual reading		Spectrophotometric reading	
	Reactive	Nonreactive	Reactive	Nonreactive
IFA (≥1:16 reactive)				
Reactive	57	0	56	1
Nonreactive	6	341	4	343
IFA (≥1:2 reactive)				
Reactive	63	1	60	4
Nonreactive	0	340	0	340

^a See Table 4 for a comparative summary of the data.

of ≥0.127. Negative control A_{610} values ranged from -0.012 to 0.013, with a mean of 0.005 ($n = 36$). Positive control A_{610} values ranged from 0.149 to 0.393, with a mean of 0.276 ($n = 36$).

DISCUSSION

All specimens received in our laboratory with a request for toxoplasmosis serology were screened by IHA unless the physician requested an IgM titer or the patient was under 1 year of age. These specimens were tested by IFA only, thus explaining the difference between the results for the 404 specimens included in the IFA comparisons and those for the 389 specimens included in the IHA comparisons.

Three laboratory workers performed with SUDS test during this study (BO, 11%; JH, 31%; and JN, 58%). Seventeen samples were repeated in all tests at the conclusion of the study to confirm previous results whenever discrepancies between tests occurred. The IHA and IFA results differed from that of the initial test for 1 of 17 samples and SUDS results differed from that of the initial test for 5 of 17 samples which were recorded as ± or 1+. These last five samples were recorded as either reactive ($n = 1$) or nonreactive ($n = 4$) based on the results of repeat testing.

Because of the insidious nature of the disease, it is extremely difficult to obtain a reliable clinical history of patients with serological evidence of toxoplasmosis. We conservatively defined reactivity at a titer of ≥1:16 in IFA as the benchmark on which to evaluate SUDS. Because SUDS has sensitivity and specificity similar to those of IFA at titers of <1:16, we also evaluated SUDS in this range. Turunen et al. (15) have demonstrated the intraocular synthesis of IgG, and the sensitivity of SUDS may have application for the diagnosis of toxoplasmic chorioretinitis or uveitis, although

TABLE 4. Comparative summary of SUDS and IFA results

Analysis	% for the following reading methods and IFA titers:			
	Visual reading		Spectrophotometric reading	
	≥1:16	≥1:2	≥1:16	≥1:2
Sensitivity	100	98.4	98.2	93.8
Specificity	98.3	100	98.8	100
Efficiency	98.5	99.8	98.8	99
Positive predictive value	90.5	100	93.3	100
Negative predictive value	100	99.7	99.7	98.8

TABLE 5. Comparative summary of IHA and SUDS as screening tests for toxoplasmosis^a

Test	%				
	Sensitivity	Specificity	Efficiency	Positive predictive value	Negative predictive value
IHA	96.4	99.4	99.0	96.4	99.4
SUDS					
Visual reading	100	98.3	98.5	90.5	100
Spectrophotometric reading	98.2	98.8	98.8	93.3	99.7

^a Diagnosis of toxoplasmosis is defined for these calculations as an IFA titer of $\geq 1:16$.

to date, SUDS has not been evaluated by using non-serum specimens.

Although we observed few false-positive SUDS results, circulating immune complexes or contaminating particulate material in serum samples may have bound to the matrix inside the SUDS cartridge, thereby causing false-positive results. Centrifugation of one specimen with bacterial contamination eliminated this problem. Repeated freezing and thawing of specimens increased the risk of false-positive results because of the formation of nonspecific immune complexes. False-positive ELISA results for toxoplasmosis caused by rheumatoid factor and antinuclear antibody have been reported, but we did not determine the susceptibility of SUDS to interfering cross-reactivities (12). The specific immunoglobulin classes that reacted by SUDS were not characterized, but IgG response presumably accounted for most activity. SUDS is intended for the detection of *T. gondii* immune status with a single serum specimen. It does not distinguish between IgM and IgG antibody, so the diagnosis of early or congenital toxoplasmosis requires quantitative testing of acute and convalescent sera or a test which specifically measures IgM antibody.

The SUDS kit contains all the necessary reagents and materials that are necessary to perform the test. The ease and speed of performance of SUDS commend it to a busy physician office laboratory. A few minor inconveniences are associated with performance of the test. The SUDS sample cup is short, and individuals with large fingers find it difficult to grasp. Care must be exercised to avoid the splashing of serum on the rim of the test cartridge or the bench top when the sample-diluent-antigen-latex mixture is poured into the

FIG. 1. Comparison between SUDS A_{610} values obtained with an experimental reader and IHA results.

FIG. 2. Comparison between SUDS A_{610} values obtained with an experimental reader and IFA results.

funnel of the cartridge. While color development was complete for most serum samples, 6 of 404 specimens were recorded as weakly reactive ($\pm$ or 1+) on the initial visual reading, and 3 of 6 were ascribed $\pm$ reactivity on repeat testing. These last three specimens were considered nonreactive for test performance calculations. The excellent agreement between visual and spectrophotometric readings of SUDS make use of the reader unnecessary.

The results of our study compare favorably with the data presented in the kit instruction booklet and those of Taylor et al. (J. M. Taylor, W. J. Payne, Jr., and K. W. Walls, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C337, p. 379). Whether the test is read visually or spectrophotometrically, the high sensitivity and negative predictive value, together with the ease and speed of performance, make SUDS a very effective screening method for detecting antibody to toxoplasmosis by using a single serum specimen.

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