

## NOTES

### Evaluation of a Rapid Screening Immunoassay for Antibodies to *Toxoplasma gondii*

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**A total of 349 human serum samples were examined for anti-*Toxoplasma* antibodies by the Murex Single Use Diagnostic System (SUDS) qualitative screening test, indirect hemagglutination assay (IHA), and indirect immunofluorescence assay (IFA). Concordant results with SUDS and IHA were obtained for 91.9% of serum samples; 8.9% were SUDS<sup>+</sup> and IHA<sup>-</sup>; none were SUDS<sup>-</sup> and IHA<sup>+</sup>. Comparison of the SUDS assay with IFA showed a concordance of 95.3%, with a sensitivity of 97.5% and a specificity of 94.7%. Moreover, the positive and negative predictive values were 84.9 and 99.2%, respectively, when results of the SUDS assay and IFA were compared. The SUDS assay is a rapid, simple test requiring no instrumentation and can be performed on 50  $\mu$ l of serum, features which make this an excellent screening test for detecting anti-*Toxoplasma* antibodies, particularly in outpatient settings.**

The value of testing for specific anti-*Toxoplasma gondii* antibodies has led to the development of a number of antibody detection methods. Among the first assays described was the highly specific Sabin-Feldman dye test (7), followed by indirect hemagglutination assay (IHA; 3), indirect immunofluorescence assay (IFA; 6, 8), enzyme-linked immunosorbent assay (ELISA; 9), and fluorescence immunoassay (10). Such methods are readily applied in a large clinical laboratory but are difficult to adapt to rapid screening applications (e.g., in physician offices, in emergency care, or in field operations). Viable *T. gondii* tachyzoites are required for the Sabin-Feldman dye test, and considerable sample manipulation is necessary for IHA (particularly if nonspecific agglutinins are present, since these samples require agglutinin adsorption and retesting). A significant investment in instrumentation must be made for the IFA, ELISA, and fluorescence immunoassay techniques. With these requirements in mind, we evaluated the performance of a commercially available Single Use Diagnostic System (SUDS) for the detection of antibodies to *T. gondii*. The test requires 50  $\mu$ l of serum, is completed in 10 min, and requires no instrumentation.

A total of 353 serum samples which had been submitted to our laboratory for *Toxoplasma* antibody studies were tested by the SUDS assay, IHA, and IFA. In accordance with the instructions of the manufacturer, the serum samples were stored at 2 to 8°C and tested within 1 week of the time of collection. In addition, samples demonstrating slight to moderate hemolysis, icterus, or lipemia were chosen for testing by the SUDS assay and by IHA. Serum samples with discordant results from the SUDS assay and IHA were also tested by IFA. To evaluate the potential for long-term storage, 28 serum samples were tested before and after one freeze (-20°C)-thaw cycle. Serum samples which developed altered reactivity were centrifuged for 3 min at 5,300  $\times$  g in a Beckman Microfuge 12 and retested.

The SUDS assay for *Toxoplasma* antibodies (Murex Corp., Norcross, Ga.) is based on an enzyme immunoassay with a solid-phase immunoadsorbent consisting of latex-bound *T. gondii* soluble antigens. Serum samples, reagents, and washes are passed over a filter in a disposable cartridge which retains the antigen-latex suspension and allows unbound reagent and wash solutions to pass through. One drop of a serum sample is mixed with 1 drop of antigen-latex suspension and incubated for 2 min. One milliliter of diluent is added and poured into the cartridge, followed by 1.5 ml of wash reagent. This is followed by a 2-min incubation with 1 drop of alkaline phosphatase conjugated to anti-human immunoglobulin G. Wash reagent (1.5 ml) is then added, followed by 2 min for color development with 1 drop of a chromogenic substrate. Color development occurs with positive serum samples in less than 2 min. Finally, 4 drops of stop reagent are added, and results are read visually. Blue is considered positive; absence of blue indicates negative. All incubations are performed at room temperature.

The IHA (Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) was performed according to the instructions of the manufacturer. The test uses stabilized sheep erythrocytes sensitized with a soluble extract of *T. gondii*. Samples with nonspecific agglutinins were adsorbed and retested. The IFA, which uses intact *T. gondii* organisms fixed to slides, was performed according to the instructions of the manufacturer (Electro-Nucleonics, Inc., Columbia, Md.).

A total of 353 random serum samples were tested by both the SUDS and IHA. However, IHA results could not be obtained for four specimens (1.1%) because of persistent nonspecific agglutinins. Of the remaining 349 serum samples, 91.9% agreement with the results of the SUDS assay was found, with 16.9% showing positive results and 74.2% showing negative results (Table 1). The remaining 31 (8.9%) of 349 serum samples that were not in agreement were SUDS<sup>+</sup> and IHA<sup>-</sup>. Of those 349 serum samples, 344 were also tested by IFA. The IFA identified 81 (23.5%) of 344 samples as positive for *Toxoplasma* antibodies in contrast to 16.9%

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TABLE 1. Results of testing random serum samples for *Toxoplasma* antibodies by the SUDS assay and IHA or IFA

Murex SUDS result <sup>a</sup>	No. of results by:			
	IHA		IFA	
	+	-	+	-
+ <sup>b</sup>	59	31	79	14
- <sup>c</sup>	0	259	2	249

<sup>a</sup> Sensitivity and specificity of the SUDS assay were 100.0 and 89.3%, respectively, compared with IHA and 97.5 and 94.7% compared with IFA.

<sup>b</sup> Predictive value of a SUDS-positive result was 65.6% compared with IHA and 84.9% compared with IFA.

<sup>c</sup> Predictive value of a SUDS-negative result was 100% compared with IHA and 99.2% compared with IFA.

positive by IHA. Comparison of the results from the SUDS assay with those from IFA shows agreement of 95.3%.

The SUDS assay achieved a sensitivity and specificity of 100.0 and 89.3%, respectively, when compared with IHA, and 97.5 and 94.7%, respectively, when compared with IFA. In the latter comparison, the predictive value of a SUDS-positive result is 84.9% and that of a SUDS-negative result is 99.2%.

Of 59 samples which were either hemolyzed, icteric, or lipemic, 10 samples were discordant (SUDS<sup>+</sup> and IHA<sup>-</sup>; Table 2). Five of these were IFA<sup>+</sup> and five were IFA<sup>-</sup> with polar staining. Thus, the condition of these samples did not appear to be incompatible with the SUDS method.

The SUDS kit manufacturer recommends using freshly collected serum which is refrigerated but not frozen. Although this requirement is reasonable for the intended purpose of the assay, the inability to store samples for extended periods can be a limitation. Therefore, 28 serum samples were collected and tested as recommended by the SUDS assay and the IHA. These samples were then frozen at -20°C, stored for 1 to 2 weeks, thawed, and retested. Reactivity was not lost after storage at -20°C. However, five serum samples for which concordant negative results were obtained with fresh samples converted to positive results in the SUDS assay after one freeze-thaw cycle. High-speed centrifugation eliminated this problem; upon repeat testing, the SUDS results with centrifuged frozen serum were identical to those obtained with fresh serum.

ELISA for detecting *Toxoplasma* antibodies has been described previously (2, 4, 9). Solid-phase materials that have been used include plastic disks (4), polystyrene microtitration plates (9), and plastic beads (2). Latex has been used as a solid phase for antigen adsorption in agglutination assays (1, 5). The application of a latex solid phase to ELISA labeling techniques is a novel approach to *Toxoplasma* serology. This stratagem has permitted the development of a rapid screening test with the sensitivity of an ELISA. The procedure identified 97.5% of serum specimens that were positive by IFA. Of those samples that were SUDS<sup>+</sup> and IHA<sup>-</sup>, a significant proportion was identified as positive by IFA, including one serum sample obtained from a 1-day old

TABLE 2. Studies with hemolyzed, icteric, and lipemic sera

Type of serum	Response by SUDS assay	No. of responses by:			
		IHA		IFA <sup>a</sup>	
		+	-	+	-
Hemolyzed	+	0	3	2	1
	-	0	16		
Icteric	+	1	5	3	2
	-	0	20		
Lipemic	+	2	2	0	2
	-	0	10		

<sup>a</sup> Results with discordant (SUDS<sup>+</sup>, IHA<sup>-</sup>) serum samples.

neonate. It appears that both the SUDS assay and IFA are distinctly more sensitive than IHA, and an excellent agreement is found when the SUDS assay is compared with IFA. Results of the assay are available within 10 min of obtaining serum specimens. Neither expensive equipment nor technical expertise is necessary to perform the test, and rapid results can be obtained from 50 µl of serum, features which make this an excellent test, particularly for outpatient settings.

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