JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1997, p. 217–222 Vol. 35, No. 1
0095-1137/97/$04.00+0

Capture-S, a Nontreponemal Solid-Phase Erythrocyte Adherence Assay for Serological Detection of Syphilis

DARRYL L. STONE,* MITCHELL C. MOHENG, SUSAN ROLIH, AND LYLE T. SINOR

Immucor, Inc., Norcross, Georgia 30071

Received 2 July 1996/Returned for modification 20 August 1996/Accepted 17 October 1996

A solid-phase erythrocyte adherence assay has been developed for the serological detection of reagin antibodies in syphilis. Capture-S (Immucor, Inc., Norcross, Ga.) is a nontreponemal, qualitative screening test for the detection of immunoglobulin G (IgG) and IgM antilipid antibodies in serum or plasma samples from blood donors. The Capture-S assay utilizes a modified Venereal Disease Research Laboratory antigen bound to microtitration wells and anti-IgG- plus anti-IgM-coated indicator erythrocytes as the detection system. The Capture-S assay was evaluated at six separate sites on 10,942 specimens. For patient samples of clinically diagnosed syphilis categories ($n = 366$), the Capture-S assay yielded a sensitivity of 80.7% versus 80.3% for the rapid plasma reagin (RPR) card test (Becton Dickinson Microbiology Systems, Cockeysville, Md.). In comparative experiments on patient and donor samples ($n = 10,222$), the Capture-S assay demonstrated a sensitivity of 94% compared to 91.2% for the RPR card test. The Capture-S and RPR card tests produced essentially equivalent specificities of 99.2% and 99.3%, respectively, for this sample population. For five test sites, the Capture-S and RPR card test demonstrated a 98.3% agreement (10,085 of 10,264) of test results. These evaluations indicate that the Capture-S compares favorably to the RPR card test in assay sensitivity and specificity, with the added benefits of ease of use, accommodation of high-volume testing, and potential for automation.

Infection with the spirochete *Treponema pallidum* subsp. *pallidum*, the etiological agent of syphilis, produces at least two types of antibodies within the human host: treponemal antibodies, which react with *T. pallidum* and other pathogenic treponemes, and nontreponemal antibodies (reagin), which react with cardiolipin, cholesterol, and lecithin antigen mixtures (1). The Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagin (RPR) antigen slide tests were originally developed to detect reaginic antibodies in these individuals (10). The serological screening of patient and donor blood samples with a nontreponemal test, such as the RPR card test, followed by confirmation of reactivity with the fluorescent treponemal antibody absorption (FTA-ABS) test is commonly used in the United States (7, 10). The RPR card test can be very labor-intensive when screening large numbers of samples and has limited potential for automation (6, 8). Enzyme-linked immunoassays (ELISAs) for syphilis screening which can accommodate high-volume testing and automation have been developed (11, 12, 17, 21, 23, 24). However, ELISA procedures require the addition of multiple reagents and multiple washing steps and can take up to 190 min to complete (21).

To address these issues, we developed a nontreponemal, qualitative screening test for the detection of reagin antibodies in serum or plasma samples from blood donors. The assay, called Capture-S, is a solid-phase erythrocyte adherence (SPEA) antibody detection system based on the procedures of Plapp et al. (15, 16). This procedure is a modification of the mixed agglutination tests for antigen and antibody detection of Coombs et al. (3) and Högman (5) employing anti-immunoglobulin G (IgG) and anti-IgG-coated erythrocytes (RBCs) as the indicator system. Currently available capture assays for the detection of antibodies to RBCs or platelets use anti-IgG-coated RBCs as the indicator system (18). Capture-S uses a modified VDRL antigen bound to microtitration wells and anti-IgG- plus anti-IgM-coated indicator RBCs as the detection system.

In this report, we describe the antigen plate preparation, assay procedure, reproducibility, clinical specificity, and automated interpretation of reactions for the Capture-S assay. Initial evaluations indicate that the Capture-S assay compares favorably to the RPR card test in assay sensitivity and specificity, with the added benefits of ease of use, accommodation of high-volume testing, and potential for automation.

MATERIALS AND METHODS

Antigen plates. Round-bottom polystyrene microtitration wells (Nunc, Roskilde, Denmark) were coated with 100 μl of a phthalocyanin dye (Sigma Chemical Co., St. Louis, Mo.) as previously described (19). The wells were washed three times with phosphate-buffered saline (PBS), consisting of 1.92 g of K_2HPO_4 (Amresco, Solon, Ohio) per liter, 0.50 g of KH_2PO_4 (Amresco) per liter, and 9.0 g of NaCl (Fisher), pH 7.2. The microtitration wells were then filled with 100 μl of VDRL antigen (Lee Labs, Grayson, Ga.) diluted 1:100 in PBS. The antigen solution was incubated in the wells overnight at 0 to 4°C. The wells were washed with PBS and then filled with 300 μl of a blocking solution containing 5% bovine serum albumin (Miles, Inc., Kankakee, Ill.). The blocking solution was aspirated from the wells, and the plates were sealed in foil pouches with dessicant (United Desiccants, Belen, N.Mex.).

Indicator RBCs. Indicator RBCs were prepared by the method of Sinor et al. (20). Briefly, Rh-positive human RBCs were washed three times with an RBC preservation solution (RPS; Immucor) to remove serum proteins. The RBCs were incubated with an equal volume of a primary-antibody coating solution containing human polyclonal IgG anti-D (Immucor) and human monoclonal IgM anti-D (Immucor) in RPS for 15 to 60 min at 37°C. The RBCs were washed with RPS to remove unbound antibodies. The primary-antibody coated RBCs were incubated for 15 to 60 min at 37°C with a secondary-antibody coating solution containing rabbit anti-human IgG (Immucor) and goat anti-human IgM (Sigma). The RBCs were washed three times with RPS and then resuspended to a hematocrit of 0.6%. The anti-IgG- plus anti-IgM-coated indicator RBCs were stored at 1 to 10°C prior to use.

Capture LISS. Capture LISS (Immucor) is a low-ionic-strength additive solution containing glycine and sodium azide, pH 7.0 (22).

Assay procedure. Prior to the start of the assay, all reagents, samples, and controls were allowed to reach room temperature. The antigen-coated microtitration wells were removed from their protective pouch, and 2 drops (100 μl) of Capture LISS were added to all test wells. One drop (50 μl) of unheated serum or plasma (EDTA or sodium citrate anticoagulant) was added to the test wells and incubated for 5 min at room temperature. Each microtitration well was then

* Corresponding author. Mailing address: Immucor, Inc., 3130 Gateway Dr., Norcross, GA 30071. Phone: (770) 441-2051. Fax: (770) 441-3807.

aspirated and washed 6 to 8 times with PBS by using a semiautomated plate washer (EL401; Bio-Tek Instruments, Winooski, Vt.). One drop (50 μl) of indicator RBCs was immediately added to each test well. The microtitration wells were then centrifuged for 2 min at 750 × *g* in a Hettich Universal centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The microtitration wells were removed from the centrifuge, and the results were scored as reactive or nonreactive based on an SPRCA scoring system previously described (22). Briefly, reactive wells were characterized by adherence of the indicator RBCs to part or all of the well surface, whereas nonreactive wells contained tight buttons of indicator RBCs at the bottom of the test well, with no area of adherence.

RPR card test. The RPR card test (Becton Dickinson Microbiology Systems, Cockeysville, Md.) was performed according to the manufacturer's insert with reagents provided by the manufacturer.

Reproducibility. To compare the reproducibility of the Capture-S assay to the RPR card test, identical panels of coded samples were provided to four laboratories participating in the Capture-S evaluations. Each participating laboratory tested the coded samples by both the Capture-S assay and the RPR card test. The reproducibility panel was constructed in a manner which followed closely the recommendations provided by the Centers for Disease Control and Prevention (2). Specifically, six serum sample pools were prepared consisting of two pools of moderate reactivity, two pools of weak reactivity, and two nonreactive pools. Each pool was divided into coded aliquots comprising a total of sixty samples. The reproducibility samples were arranged in sets of six samples in an order that allowed data to be obtained on within-day and day-to-day reproducibility. Each trial site tested a set of six reproducibility samples on each of 10 testing days. The first five testing days (the first 30 reproducibility samples) at each site were consecutive in order to detect short-term error. The remaining five testing days were distributed throughout the remainder of the clinical trial period in order to detect long-term error.

Clinical specificity. Clinical testing to determine the specificity of the Capture-S assay was performed on samples derived from patients with documented diseases and conditions other than syphilis that have been associated with false-positive reactions. Both the Capture-S assay and the RPR card test were used to test specimens from the following categories: systemic lupus erythematosus and/or anti-nuclear antibody (anti-DNA) positive, drug addicts, post-hepatitis B virus vaccination specimens, *Legionella*-positive, Lyme disease, infectious mononucleosis, mycoplasma-positive, multiple myeloma specimens, pregnancy, rheumatoid factor-positive, and rubella-positive specimens. The disease status or clinical condition of the samples in each category was verified by clinical diagnosis and/or test results from commercially available assays.

Automated interpretation of Capture-S reactions. To determine if the SPRCA reactions of the Capture-S assay could be read by a microplate reader with appropriate interpretative software, 1,076 donor samples were tested at Immucor, and the visual interpretations of the test reactions were compared to the results produced by an Inverness Blood Group Systems (IBG Systems, Inc., West Sussex, England) Multireader Plus microplate reader equipped with ScreenTest interpretative software. In SPRCA testing, a negative reaction has a peripheral area which allows high light transmission and a central button of cells which allows low light transmission. In contrast, a positive reaction is characterized by a uniform distribution of cells and a correspondingly uniform optical transmission profile. In the ScreenTest program, an interpretation of positive (+), questionable (?), or negative (−) is based on the assessment of reaction strength made by a multistage algorithm and the comparison of the reaction strength with positive and negative thresholds set in the antibody screening test definition. For the Immucor in-house comparison of manual and automated reading of Capture-S reactions, all test wells underwent both visual and automated interpretations of results.

Clinical evaluation of Capture-S. The performance of the Capture-S assay was evaluated by in-house testing of blood donor samples and at five external test sites. The sensitivity and specificity values at each site were calculated by the method of Galen and Gambino (4), utilizing the confirmatory test results as the true reactive status.

(i) Site 1. Prior to implementation of parallel studies at clinical trial sites, the Capture-S assay was evaluated at the Centers for Disease Control and Prevention (CDC), Treponemal Pathogenesis and Immunobiology Branch, Atlanta, Ga. The CDC staff performed an evaluation of the Capture-S assay on 720 serum bank samples consisting of 366 samples from different syphilis categories and 354 presumed nonsyphilitic samples. The syphilis samples were categorized by disease stage and included samples from patients diagnosed with primary, secondary, latent, and unknown stages of disease. The samples in each syphilis category included serum samples collected prior to treatment and samples collected at approximately 6 and 12 months after treatment was initiated. All samples were tested by Capture-S, the RPR card test, and a microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP) (Miles Diagnostics Division, Elkhart, Ind.). Additionally, selected samples were also tested by a fluorescent treponemal antibody absorption (FTA-ABS) confirmatory assay (Zeus FTA-ABS; Wampole Laboratories, Cranbury, N.J.).

(ii) Site 2. A parallel evaluation of the Capture-S assay and RPR card test was performed at site 2 on 1,917 serum samples submitted for routine syphilis screening. Site 2 routinely tests a general population equivalent to a public health laboratory specimen population having an average reactivity of approximately 3 to 10% in the RPR card test. The samples tested included specimens from patients exhibiting clinical symptoms of syphilis infection, patients being monitored for treatment of syphilis, intravenous drug abusers, and human immunodeficiency virus-infected individuals. All reactive sera were tested by an FTA-ABS confirmatory assay (Wampole).

(iii) Site 3. Site 3 was a laboratory testing an adult blood donor population characteristic of the midwestern region of the United States. A total of 1,932 blood donor samples were tested at site 3. Only serum samples were tested at this site, and all samples were loaded onto Capture-S microtiter plates by an automated liquid handler (FPC-II; Abbott Diagnostics, Abbott Park, Ill.). All reactive sera were tested by an FTA-ABS confirmatory assay (Wampole).

(iv) Site 4. Site 4 was a plasma center that routinely tests an adult population which donates plasma samples for commercial purposes. For the Capture-S clinical evaluation, paired serum and plasma samples (plasma samples treated with sodium citrate [anticoagulant]) obtained at the time of donation from each of 1,193 donors were tested by Capture-S and a RPR card test. Only serum samples were tested by the RPR card test, whereas both serum and plasma (sodium citrate-treated) samples were tested by Capture-S. All reactive sera were tested by a FTA-ABS confirmatory assay (Wampole).

(v) Site 5. A total of 1,425 blood donors were tested at site 5. This site was a laboratory routinely testing a blood donor population characteristic of the southern region of the United States. Paired serum and plasma samples (plasma samples treated with EDTA [anticoagulatant]) obtained at the time of donation were tested by the Capture-S assay. Only serum samples were tested by the RPR card test. Reactive sera were tested by the FTA-ABS confirmatory assay (Wampole). All test samples were loaded onto the Capture-S microtiter plates by an automated liquid handler (FPC-II; Abbott Diagnostics).

RESULTS

Preliminary evaluation of Capture-S. Initial evaluation of the Capture-S assay was performed in-house on 1,179 EDTA-treated donor samples, and the results were compared with those obtained with the RPR card test. Reactive samples were tested by the MHA-TP (Miles) assay, and sensitivity and specificity values were calculated based on the screening test results compared with the MHA-TP confirmatory test result (Table 1). In the comparative study, neither assay produced a false-nonreactive result, which equated to a sensitivity of 100% for both tests. The Capture-S assay exhibited a specificity of 99.1% (1,153 of 1,163) versus 99.6% (1,158 of 1,163) for the RPR card test. The five donor samples which produced false-reactive results in the RPR card test were also reactive in the Capture-S assay. The five additional Capture-S false-reactive results were responsible for the 0.5% lower specificity in the Capture-S assay. The Capture-S and RPR card tests demonstrated an initial testing agreement of 98.3% (1,159 of 1,179). After repeat testing of discrepant samples, the Capture-S and RPR card test demonstrated a 99.6% (1,174 of 1,179) agreement.

Clinical evaluation of Capture-S. The Capture-S assay was evaluated on clinical specimens at five external test sites as described in Materials and Methods. At each site, the Capture-S assay was compared to the RPR card test which was the standard screening test routinely used at that facility. The FTA-ABS confirmatory test was performed on all reactive samples. Sensitivity and specificity values were calculated based on the screening test results compared with the FTA-ABS confirmatory test result. A summary of the data from these external evaluations is given in Table 1.

(i) Site 1. At the CDC, the clinical sensitivities of the Capture-S and RPR card tests were compared by testing 366 reactive syphilis samples from treated and untreated patients at primary, secondary, latent, and unknown stages of the disease. A summary of the comparative study is presented in Table 2. In the primary syphilis category, testing on 29 specimens from untreated patients produced three false-nonreactive results with the Capture-S assay compared to one false-nonreactive result with the RPR card test. The Capture-S and RPR card tests had sensitivities of 88.5 and 96.2%, respectively, in this untreated group. In contrast, the Capture-S assay produced nine fewer false-nonreactive results than the RPR card test on 54 primary syphilis samples from treated patients. The Cap-

TABLE 1. Results of serological evaluation of Capture-S assay

Site and test[a]	No. of samples giving the following result:				Total no. of samples	Sensitivity (%)	Specificity (%)
	True reactive	True nonreactive	False reactive	False nonreactive			
In-house							
Cap-S	16	1,153	10	0	1,179	100	99.1
RPR	16	1,158	5	0	1,179	100	99.6
Site 1							
Cap-S	280	357	14	69	720	80.2	96.2
RPR	278	366	5	70	719	79.9	98.7
Site 2							
Cap-S	226	1,621	21	13	1,881	94.6	98.7
RPR	225	1,611	31	14	1,881	94.1	98.1
Site 3							
Cap-S	1	1,917	14	0	1,932	100	99.3
RPR	1	1,921	10	0	1,932	100	99.5
Site 4							
Cap-S	30	2,320	34	2	2,386	93.8	98.6
RPR	7	1,174	3	9	1,193	43.8	99.7
Site 5							
Cap-S	10	2,828	4	2	2,844	83.3	99.9
RPR	6	1,414	2	0	1,422	100	99.9

[a] Abbreviations: Cap-S, Capture-S; RPR, RPR card test.

ture-S assay had a sensitivity of 62.5% versus 43.8% for the RPR card test in this treated group. Combining the results from all primary syphilis samples reveals that the Capture-S assay produced seven fewer false-nonreactive results than the RPR card test and yielded a Capture-S sensitivity of 71.6% compared to 62.2% for the RPR card test. In the secondary syphilis category, the Capture-S assay produced five more false-nonreactive results (three false nonreactives in the untreated group and two false nonreactives in the treated group on 139 samples) than the RPR card test. The Capture-S assay demonstrated a sensitivity of 84.4% versus 88.1% for the RPR card test in this secondary syphilis group. In the latent syphilis category, the Capture-S assay produced three more false-nonreactive (four more false nonreactives than RPR in the treated group but one less false nonreactive in the untreated group) and three more false-reactive results (one more in untreated and two more in treated group) than the RPR card test. For all latent syphilis samples, the Capture-S assay had a sensitivity of 81.7% versus 84.3% for the RPR test. In the unknown syphilis category, the Capture-S assay had two less false-nonreactive results than the RPR card test, which is reflected in the higher sensitivity of 82.6% compared to 73.9% for the RPR test.

TABLE 2. Capture-S and RPR testing of syphilis serum bank samples

Syphilis category	Total no. of samples	Capture-S					RPR card test				
		No. of samples giving the following result[a]:				Sensitivity (%)	No. of samples giving the following result:				Sensitivity (%)
		TR	TN	FR	FN		TR	TN	FR	FN	
Primary											
Untreated	29	23	3	0	3	88.5	25	3	0	1	96.2
Treated	54	30	4	2	18	62.5	21	6	0	27	43.8
Secondary											
Untreated	50	45	0	1	4	91.8	47	0	1	1	97.9
Treated	89	69	1	2	17	80.2	71	0	3	15	82.6
Latent											
Untreated	41	37	0	2	2	94.9	36	1	1	3	92.3
Treated	80	57	2	2	19	75.0	61	4	0	15	80.3
Unknown											
Untreated	12	10	0	0	2	83.3	9	0	0	3	75.0
Treated	11	9	0	0	2	81.8	8	0	0	3	72.7
Totals	366	280	10	9	67	80.7	278	14	5	68	80.3

[a] Abbreviations: TR, true reactive; TN, true nonreactive; FR, false reactive; FN, false nonreactive.

TABLE 3. Capture-S results on paired serum and plasma samples

Site and sample	No. of samples giving the following result:				Total no. of samples	Sensitivity (%)	Specificity (%)	Correlation (%)
	True reactive	True nonreactive	False reactive	False nonreactive				
Site 4								
Serum	15	1,160	17	1	1,193	93.8	98.6	100
Plasma (sodium citrate)	15	1,160	17	1	1,193	93.8	98.6	
Site 5								
Serum	5	1,414	2	1	1,422	83.3	99.9	100
Plasma (EDTA)	5	1,414	2	1	1,422	83.3	99.9	

A summation of the results of all untreated samples in the syphilis categories (132 samples) reveals that the Capture-S assay produced 3 more false nonreactives (11 false nonreactives for Capture-S and 8 false nonreactives for RPR) and one more false-reactive result (3 false reactives for Capture-S and 2 false reactives for RPR) than the RPR card test. These results produced a Capture-S sensitivity of 91.3% and an RPR sensitivity of 93.6% for untreated syphilis samples. The 2.3% lower sensitivity of the Capture-S assay in the untreated syphilis group can be attributed to both the few additional false-nonreactive results in the primary and secondary syphilis categories and the small sample population tested. In contrast to the untreated syphilis group, a summation of the results of all treated samples in the syphilis categories (234 samples) yields a Capture-S assay sensitivity of 74.7% versus 72.9% for the RPR card test. The 1.8% increased sensitivity of the Capture-S assay over the RPR card test in the treated syphilis category can be mainly attributed to fewer false-nonreactive results in the primary syphilis category. A compilation of the test results of all samples in the syphilis categories (treated and untreated samples; 366 samples) yields a Capture-S sensitivity of 80.7% versus an RPR sensitivity of 80.3%. An examination of the specificity of each test in the nonsyphilis sample group (354 samples) reveals a 2.4% lower specificity of the Capture-S assay (98.6% Capture-S specificity versus 100% RPR specificity) compared to the RPR card test due to five false-reactive results (Table 1).

A summary of the results of all samples tested in the CDC evaluation (720 samples) demonstrates that the Capture-S assay and the RPR card test gave similar results. For the entire study (Table 1), the Capture-S assay demonstrated a sensitivity of 80.2% compared to 79.9% for the RPR card test. The Capture-S assay had a specificity of 96.2% for the entire CDC evaluation versus 98.7% for the RPR card test. The 2.5% lower specificity of the Capture-S assay can be attributed to nine additional false-reactive results occurring primarily in the nonsyphilis and treated syphilis sample groups.

(ii) Site 2. The testing of a public health sample population at site 2 produced a Capture-S sensitivity of 94.6% compared to 94.1% for the RPR card test. The 0.5% higher sensitivity of the Capture-S assay was due to one less false-nonreactive result (13 Capture-S false nonreactives versus 14 RPR false nonreactives) than the RPR card test. Additionally, the Capture-S assay demonstrated a specificity of 98.7% compared to 98.1% for the RPR card test due to 10 fewer false-reactive results. The Capture-S and RPR card tests demonstrated an initial testing agreement of 93.6% (1,794 of 1,917). After repeat testing of discrepant samples, the Capture-S and RPR card tests demonstrated a 96% (1,841 of 1,917) agreement.

(iii) Site 3. After 4 weeks of Capture-S and RPR testing on 1,924 blood donor samples at site 3, no true-reactive samples were encountered in the clinical trials. At that time, the Capture-S assay had a specificity of 99.6% compared to 99.8% for the RPR card test (data not shown). In order to determine assay sensitivity, technologists at site 3 collected only reactive samples for an additional 17 days. Eight donor samples which were reactive by both Capture-S and the RPR card test were collected. Following FTA-ABS confirmatory testing, only one sample was classified as a true-reactive sample, with the remaining seven samples producing false-reactive results. Thus, the Capture-S assay demonstrated a specificity of 99.3% compared to 99.5% for the RPR card test on 1,932 donor samples. Both the RPR card test and Capture-S demonstrated a sensitivity of 100% on one true-reactive sample encountered during the clinical trials. The Capture-S and RPR card tests demonstrated an initial testing agreement of 98.6% (1,905 of 1,932). After repeat testing of discrepant samples, the Capture-S and RPR card test demonstrated a 99.8% (1,928 of 1,932) agreement.

(iv) Site 4. As indicated in Table 3, clinical data obtained from site 4 demonstrates that there is no difference in sample reactivity or assay performance between serum and plasma (sodium citrate-treated) samples in the Capture-S assay. A 100% correlation of test results was obtained between the paired serum and plasma (sodium citrate) samples. Results from this site indicate a Capture-S specificity of 98.6% for both serum and plasma (sodium citrate) samples (due to 17 false-reactive results from both serum and plasma [sodium citrate] samples of 1,193 donors tested) compared to 99.7% for the RPR card test (Table 1). However, the Capture-S assay demonstrated a sensitivity of 93.8% for both serum and plasma (sodium citrate) samples compared to 43.8% for the RPR card test (due to 9 false-nonreactive results) in this sample population. The Capture-S and RPR card tests demonstrated an initial testing agreement of 96.6% (2,306 of 2,386). After repeat testing of discrepant samples, the Capture-S and RPR card tests demonstrated a 97.7% (2,330 of 2,386) agreement.

(v) Site 5. Comparative data obtained from site 5 demonstrates a 100% correlation of sample reactivity between paired serum and plasma (treated with EDTA) samples in the Capture-S assay (Table 3). This data includes a 100% correlation of results between individual paired donor samples. Results from the site (Table 1) indicate a Capture-S specificity of 99.9% for both serum and plasma (EDTA) samples (due to two false-reactive results from both serum and plasma [EDTA] samples of 1,422 donor tested). The RPR card test also demonstrated a 99.9% specificity due to two false-reactive results on 1,422 serum samples. The Capture-S assay demonstrated a

TABLE 4. Clinical specificity testing

Sample category[a]	No. of samples	No. of samples with the following result[b]:			
		R by Capture-S		NR by Capture-S	
		R by RPR	NR by RPR	R by RPR	NR by RPR
ANA(+); anti-DNA(+)	10				10
Drug addicts	10		1[c]		9
Anti-HBs(+)	10				10
Legionella(+)	10			1[d]	9
Lyme disease	10				10
Mononucleosis(+)	10				10
Mycoplasma(+)	10	1[c]		1[d]	8
Multiple myeloma	10				10
Pregnancy	10				10
RF(+)	10			1[d]	9
Rubella	10				10
Total	110	1	1	3	105

[a] Abbreviations: ANA(+), samples positive for anti-nuclear antibody; anti-DNA(+), samples positive for anti-DNA antibody; anti-HBS(+), post-hepatitis B virus vaccination specimens; (+), positive; RF(+), rheumatoid factor positive.
[b] Abbreviations: R, reactive; NR, nonreactive.
[c] Reactive by MHA-TP.
[d] Nonreactive by MHA-TP.

sensitivity of 83.3% for both serum and plasma (EDTA) samples compared to 100% for the RPR card test. The lower Capture-S sensitivity was due to one donor who produced false-nonreactive results with both serum and plasma (EDTA) samples. The Capture-S and RPR card tests demonstrated an initial testing agreement of 98.1% (2,795 of 2,850) at site 5. After repeat testing of discrepant samples, the Capture-S and RPR card tests demonstrated a 98.7% (2,812 of 2,850) agreement at this site.

Reproducibility. For four Immucor-sponsored test sites participating in the reproducibility study (sites 2, 3, 4, and 5), the Capture-S and RPR card tests demonstrated a 96.25% agreement (231 of 240 samples) of reproducibility panel test results. All discrepant reproducibility testing results occurred with the RPR card test. At site 2, the RPR card test produced seven false-reactive results of 12 samples from a nonreactive serum pool. The RPR card test demonstrated poor within-day and day-to-day reproducibility at this site on the nonreactive pool. Similarly, at site 4, two false-nonreactive results in the RPR card test were obtained on aliquots of a weakly reactive pool which had been correctly interpreted the day before and the day after the false-nonreactive results. Seven of the nine discrepant RPR card test results occurred at site 2, which was a public health laboratory. In contrast to the RPR card test results, the Capture-S assay demonstrated 100% reproducibility on all panel samples tested. The Capture-S assay demonstrated 100% agreement between all test sites. In addition, the Capture-S assay produced 100% within-day and day-to-day reproducibility at each test site. No short-term or long-term error was demonstrated for the Capture-S assay at any of the test sites.

Clinical specificity. The results of clinical specificity testing showed a 96.2% agreement (101 of 105) between the Capture-S assay and the RPR card test (Table 4). The Capture-S assay demonstrated a specificity of 100% on specimens from the clinical categories tested. In contrast, the RPR card test produced one false-nonreactive and three false-reactive results on these same specimens, as determined by MHA-TP testing. Thus, the RPR card test demonstrated a specificity of 97.2% for the clinical specimens tested in this study.

Automated interpretation of Capture-S reactions. As indicated in Table 5, the Multireader Plus with the ScreenTest program agreed with the visual interpretation of Capture-S reactions in 98.0% (1,055 of 1,076) of all wells tested. Using the visual interpretation of the reactions as the true Capture-S reactive status, the microplate reader correctly interpreted 99.4% (1,032 of 1,038) of the nonreactive wells. The six discrepant reactions were interpreted as questionable by the microplate reader. In contrast, the reader correctly interpreted 60.5% (23 of 38) of the reactive wells as positive. The remaining 39.5% (15 of 38) of the reactive wells were interpreted as questionable by the reader. More importantly, no reactive wells were misinterpreted as negative by the microplate reader. The screening test thresholds used to discriminate the solid-phase reactions were set to interpret borderline negative and weak reactions as questionable. This allows the discrimination and interpretation of weak reactions to be made by the technologist performing the assay and not the microplate reader.

DISCUSSION

We have described the initial evaluation of the Capture-S assay, a nontreponemal, qualitative screening test for the detection of IgG and IgM antilipid antibodies in serum or plasma samples from blood donors. The Capture-S assay utilizes an SPEA format which offers many advantages over flocculation and ELISA procedures. In contrast to other syphilis screening assays (10, 14, 23, 24), Capture-S allows testing of serum and plasma (EDTA or sodium citrate anticoagulant) samples. The SPEA assay requires fewer assay steps and plate manipulations, such as washing, than traditional ELISAs. The addition of a low-ionic-strength solution with the samples in the test wells allows a 5-min sample incubation compared to 30- to 90-min incubations commonly used in ELISAs. The entire Capture-S procedure can be completed in under 15 min compared to ELISA test procedures lasting from 60 to 190 min (11, 21, 23, 24). Utilizing an automated liquid handler for reagent and sample addition, an automated plate washer, and a microplate reader for interpretation of test results, one technologist can process a 96-well microtiter plate in under 22 min, or more than 1,300 samples in an 8-h work shift. Unlike flocculation and ELISAs, the Capture-S test reactions are stable and can be read and reread for up to 48 h after testing if the wells are covered and stored at 1 to 10°C.

The Capture-S assay was evaluated at six separate test sites on 10,942 specimens. A summation of the data from all sites produced a Capture-S sensitivity of 86.7% compared to 85.1% for the RPR card test. The reduced sensitivity calculated for both test methodologies can be attributed to the false-nonreactive results obtained in the CDC evaluation on frozen syphilis samples (69 false nonreactives for Capture-S and 70 false nonreactives for RPR) and the public health laboratory eval-

TABLE 5. Automated interpretation of Capture-S reactions

Visual interpretation	No. of reactions with the following result by microplate reader:			Total no. of reactions
	Positive	Negative	Questionable	
Reactive	23	0	15	38
Nonreactive	0	1,032	6	1,038
Total	23	1,032	21	1,076

uation at site 2 (13 false nonreactives for Capture-S and 14 false nonreactives for RPR). The Capture-S assay is not intended for use in public health or sexually transmitted disease laboratories. Site 2 was chosen as a Capture-S clinical trial site because of the large number of FTA-ABS reactive samples tested and because the sample population included disease states and conditions known to create problems for syphilis screening assays. An examination of the results from the donor and plasma centers reveals that the Capture-S assay produced four false-nonreactive results from 8,341 samples tested compared to nine false-nonreactive results from 4,550 samples tested with the RPR card test. Excluding data on frozen syphilis samples at the CDC, the Capture-S assay demonstrated a sensitivity of 94% compared to 91.2% for the RPR card test on patient and donor samples. Testing at these sites produced an initial testing agreement of 97.0% (9,959 of 10,264) for the Capture-S and RPR card tests. After repeat testing of discrepant samples, the Capture-S and RPR card tests demonstrated a 98.3% agreement (10,085 of 10,264).

Biological false-positive results for nontreponemal and treponemal antibody tests can occur at a rate as high as 4% in low-risk adult populations (9, 13). Autoimmune diseases, narcotic addiction, and pregnancy are the most common causes of nonspecificity for nontreponemal tests in this population. For the patient and donor populations tested in this evaluation, the Capture-S and RPR card tests demonstrated essentially equivalent specificities of 99.2% and 99.3%, respectively. However, testing of potential biological false-positive samples (Table 4) indicated that Capture-S is more specific than the RPR card test for samples from these clinical categories. The Capture-S assay also proved to be more reproducible than the RPR card test on a panel of coded samples provided to the test sites. The reproducibility panel results may reflect the easier readability of SPEA reactions compared to flocculation tests for weakly reactive and nonreactive results. The accuracy and reproducibility of the RPR card test are highly dependent on the training and experience of the technologist performing the assay, since the results are read subjectively and recorded manually. To reduce potential variability in the Capture-S assay, two test sites utilized automated liquid handlers to dispense samples into the test wells. In addition, the objective reading of Capture-S reactions using a microplate reader with interpretative software was compared to the visual interpretations of 1,076 donor sample reactions. The microplate reader correctly interpreted 99.4% of the nonreactive wells and did not produce any false-negative interpretations of reactive wells. Future software changes may improve the 98.0% concordance of visual and automated interpretation of test results.

In summary, we have developed a nontreponemal, qualitative syphilis screening test which is easy to perform, can accommodate testing of large numbers of samples, and can be automated. The sensitivity and specificity calculations for the evaluation test results along with the reproducibility and clinical specificity data demonstrate that the Capture-S assay is safe and effective and performs as well as the RPR card test for the serological detection of reagin in serum or plasma samples from blood donors with syphilis.

REFERENCES

1. **Cannefax, G. R., L. C. Norins, and E. J. Gillespie.** 1967. Immunology of syphilis. Annu. Rev. Med. **18:**471–482.
2. **Center for Disease Control.** 1977. Guidelines for evaluation and acceptance of new syphilis serology tests for routine use. U.S. Department of Health, Education, and Welfare, Atlanta, Ga.
3. **Coombs, R. R. A., J. Marks, and D. Bedford.** 1956. Specific mixed agglutination: mixed erythrocyte-platelet anti-globulin reactions for the detection of platelet antibodies. Br. J. Haematol. **2:**84–93.
4. **Galen, R. S., and S. R. Gambino.** 1975. Beyond normality: the predictive value and efficiency of medical diagnosis, p. 10–14. John Wiley & Sons, New York.
5. **Högman, C.** 1959. The principle of mixed agglutination applied to tissue culture systems. Vox Sang. **4:**12–20.
6. **Larsen, S. A.** 1981. Syphilis serology: 40 million assays annually spark research for newer diagnostic tests, p. 53–57. *In* L. Karnaugh (ed.), Lab world. North American Publishing Co., Philadelphia, Pa.
7. **Larsen, S. A., and L. L. Bradford.** 1986. Serodiagnosis of syphilis, p. 425–434. *In* N. R. Rose, H. Friedman, and J. R. Fahey (ed.), Manual of clinical lanboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
8. **Larsen, S. A., E. A. Hambie, and D. D. Cruce.** 1987. Review of the standard tests for syphilis and evaluation of a new commercial ELISA, the syphilis Bio-Enzabead test. J. Clin. Lab. Anal. **1:**300–307.
9. **Larsen, S. A., E. A. Hambie, D. E. Pettit, M. W. Perryman, and S. J. Kraus.** 1981. Specificity, sensitivity, and reproducibility among the fluorescent treponemal antibody-absorption test, the microhemagglutination assay for *Treponema pallidum* antibodies, and the hemagglutination treponemal test for syphilis. J. Clin. Microbiol. **14:**441–445.
10. **Larsen, S. A., E. F. Hunter, and S. J. Kraus (ed.).** 1990. Diagnostic tests, p. 1–26. *In* A manual of tests for syphilis. American Public Health Association, Washington, D.C.
11. **Nayar, N., and J. M. Campos.** 1993. Evaluation of the DCL Syphilis-G enzyme immunoassay test kit for the serologic diagnosis of syphilis. Am. J. Clin. Pathol. **99:**282–285.
12. **Pedersen, N. S., O. Ørum, and S. Mouritsen.** 1987. Enzyme-linked immunosorbent assay for detection of antibodies to the Venereal Disease Research Laboratory (VDRL) antigen in syphilis. J. Clin. Microbiol. **25:**1711–1716.
13. **Peter, C. R., M. A. Thompson, and D. L. Wilson.** 1979. False-positive reactions in the Rapid Plasma Reagin-Card, Fluorescent Treponemal Antibody-Absorbed, and Hemagglutination Treponemal Syphilis Serology tests. J. Clin. Microbiol. **9:**369–372.
14. **Pettit, D. E., S. A. Larsen, P. S. Harbec, J. C. Feeley, C. E. Parham, D. D. Cruce, E. A. Hambie, and M. W. Perryman.** 1983. Toluidine red unheated serum test, a nontreponemal test for syphilis. J. Clin. Microbiol. **18:**1141–1145.
15. **Plapp, F. V., W. L. Bayer, M. L. Beck, L. T. Sinor, and W. M. Coenen.** August 1986. Testing for a blood group immunological reaction. U.S. patent 4,608,246.
16. **Plapp, F. V., L. T. Sinor, J. M. Rachel, M. L. Beck, W. M. Cohen, and W. L. Bayer.** 1984. A solid phase antibody screen. Am. J. Clin. Pathol. **82:**719–721.
17. **Pope, V., E. F. Hunter, and J. C. Feeley.** 1982. Evaluation of the microenzyme-linked immunosorbent assay with *Treponema pallidum* antigen. J. Clin. Microbiol. **15:**630–634.
18. **Sinor, L. T.** 1992. Advances in solid-phase red cell adherence methods and transfusion serology. Transfus. Med. Rev. **6:**26–31.
19. **Sinor, L. T., R. Eatz, F. V. Plapp, and D. L. Stone.** October 1990. An article for performing immunological assays utilizing organic dyes and methods for producing and utilizing same. U. S. patent 4,963,478.
20. **Sinor, L. T., J. M. Rachel, and F. V. Plapp.** March 1989. Solid phase indicator blood cells and method. U. S. patent 4,816,413.
21. **Stevens, R. W., and M. E. Schmitt.** 1985. Evaluation of an enzyme-linked immunosorbent assay for treponemal antibody. J. Clin. Microbiol. **21:**399–402.
22. **Stone, D. L., R. A. Eatz, S. D. Rolih, S. J. Farlow, G. S. Hudson, and L. T. Sinor.** 1990. Red cell antibody identification by solid phase red cell adherence utilizing dried RBC monolayers. Immunohematology **6:**12–17.
23. **White, T. J., and S. A. Fuller.** 1989. Visuwell Reagin, a non-treponemal enzyme-linked immunosorbent assay for the serodiagnosis of syphilis. J. Clin. Microbiol. **27:**2300–2304.
24. **Young, H., A. Moyes, A. McMillan, and D. H. H. Robertson.** 1989. Screening for treponemal infection by a new enzyme immunoassay. Genitourin. Med. **65:**72–78.