Capture®-S

Solid Phase System for the Serological Detection of Syphilis in Blood Donors

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IMMUCOR GAMMA®

Intended Use:
Immucor's Capture-S is a nontreponemal, in vitro qualitative screening test for the detection of IgG and IgM antilipid antibodies in the serum or plasma of blood donors.

Summary of the Test:
Infection with the spirochete *Treponema 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 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 card tests were originally developed to detect antilipid antibodies in these individuals.[2] The Capture-S test is a solid phase system utilizing a modified VDRL antigen bound to microtiter wells.

Principle of the Test:
The Capture-S test consists of a modified VDRL antigen bound to microtitration wells. The wells are dried and supplied to users along with necessary reagents and controls.

The assay is a two step test carried out in microtitration wells coated with a modified VDRL antigen. In the first step, serum or plasma samples are added to the lipid-coated wells. The wells are incubated for five minutes, during which specific antibodies bind to the immobilized lipids. Unbound immunoglobulins are washed from the wells and replaced with a suspension of anti-IgG + anti-IgM-coated indicator red cells. Centrifugation brings the indicator red cells in contact with antibodies bound to the immobilized lipids. In the case of a reactive test, the migration of the indicator red cells to the bottom of the well is impeded as anti-IgG and anti-IgM bridges are formed between the indicator red cells and the lipid-bound antibodies. As a consequence, the indicator red cells adhere over the surface of the microtitration well. In contrast, in the absence of lipid antigen-antibody interactions (ie, a nonreactive test) the indicator red cells are not impeded during their migration, and pellet to the bottom of the well as a tightly packed, well-defined cell button.

Reagents:
Capture-S Microtitration Wells: Rigid U-bottom microtitration wells to which lipid antigens have been bound. The wells are enclosed in foil pouches to which a desiccant and a moisture indicator have been added. Each microtitration well is ready to be used as supplied. Store the wells at 1-30 C. If the humidity indicator enclosed within each pouch shows the presence of moisture (by the humidity indicator turning from blue to pink), the wells should not be used. Unused microtitration wells, desiccant and humidity indicator should be immediately resealed within the foil pouch to prevent the uptake of moisture. Carefully reseal the pouch to minimize the leakage of moisture into the pouch during storage. Microtitration wells resealed in a pouch should be used within two weeks (but not beyond the expiration date) provided the humidity indicator does not show the presence of moisture. **Microtitration wells removed from pouches should be used within one (1) hour.**

Adjunct Reagents to Capture Test Wells:
(Purchased separately)

Capture-S Indicator Red Cells: A suspension of human red blood cells coated with rabbit anti-human IgG and goat anti-human IgM molecules. The red blood cells (<1%) are suspended in a buffered solution to which chloramphenicol (0.25 mg/mL), neomycin sulfate (0.1 mg/mL) and gentamycin sulfate (0.05mg/mL) have been added as preservatives. Store at 1-10 C. Do not freeze.

Capture-S Reactive Control Serum: Heat-inactivated human serum containing IgG antibodies to *Treponema pallidum*. The Capture-S Reactive Control is manufactured to represent the reactivity obtained with weakly reactive donor samples. Weakly reactive samples have a titration endpoint of 1:2 or less. The Capture-S Reactive Control is at a potency that is below 2.5 standard deviations from the mean of the reactive sample population. Sodium azide (0.1%) has been added as a preservative.* Store at 1-10 C.

Capture-S Nonreactive Control Serum: Containing no antibodies to *Treponema pallidum*. Contains sodium azide (0.1%)* as a preservative.* Store at 1-10 C.

Capture LISS: A low ionic strength solution containing glycine, bromcresol purple dye and the preservative sodium azide (0.1%)*. Store at 1-10 C.

The in-date components (Capture-S test wells, Capture LISS, Capture-S Control Sera, Capture-S Indicator Red Cells) used to perform Capture-S assays can be used interchangeably with other components, irrespective of their lot numbers, provided the components are within their expiration dates.

Precautions:
For in vitro diagnostic use.

*This reagent contains 0.1% sodium azide and is classified as Harmful (Xn). R22 Harmful if swallowed.

Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into the sink, flush with a large volume of water to prevent azide build-up.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

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Donor specimens and all materials coming into contact with them should be handled as if transmitting infection and disposed of with proper precautions. Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterwards. Never pipette by mouth and avoid contact with skin and mucous membranes. Avoid splashing or forming an aerosol.

Dispose of all specimens and materials used to perform the test as if they contained infectious agents. Dispose of all waste in accordance with state and local laws.

Avoid microbial contamination of reagents or incorrect results may occur. Use aseptic technique. ▲ Store all assay components at their proper temperatures when not in use.

Key:
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Cross-contamination of samples can cause falsely positive results. Use clean pipette tips for each sample to eliminate contamination/carryover with the previous specimen.

Bring all refrigerated Capture-S assay components to room temperature (18-30 C) before testing.

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Do not use reagents beyond their expiration dates. ▲

The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

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Do not allow wells to dry once assay has begun. Do not reuse test wells.

A nonreactive result will occur if a specimen is inadvertently not added to a test well.

Incubation times or temperatures other than those specified may give erroneous results.

Store saline for washing in a clean container to avoid contamination.

▲ Store Capture-S Microtitration Wells at 1-30 C.

Specimen Collection and Preparation:

Draw a blood specimen using an acceptable phlebotomy technique. Serum or plasma (EDTA or sodium citrate anticoagulants) may be used in this assay. Testing should be performed as soon as possible to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the specimen. Should delays in testing occur, specimens should be stored at 1-10 C for up to 10 days. Alternatively, serum can be separated from red cells and stored frozen. Specimens should not be stored at room temperature (22-25 C) for more than 5 days before testing. Weakly reactive antibodies may deteriorate and become undetectable in samples stored at room temperature for several days or in samples stored for prolonged periods at 1-10 C. No interference in assay performance was detected with samples containing up to 20 mg/dL total bilirubin, 500 mg/dL hemoglobin, 10,500 mg/dL IgG, 3,800 mg/dL IgM or 2,300 mg/dL total lipid.

Do not use samples drawn into tubes with neutral gel separators. False-positive results may occur with such samples.

Procedure:

Materials Provided:

1. Capture-S Microtitration Wells in sealed pouches

Additional Reagents:

1. Capture-S Indicator Red Cells in dropper vials
2. Capture-S Reactive Control Serum in dropper vials
3. Capture-S Nonreactive Control Serum in dropper vials
4. Capture LISS in dropper vials

Additional materials required:

1. Automatic or manual microtitration plate washer, vacuum source, and trap; or hand-held multi-channel refilling syringe. Note: The Capture-S system has been designed so that automated microtitration plate washers may be incorporated in washing steps to reduce the risks associated with handling human samples.
2. Centrifuge, rotor and carriers capable of accommodating microtitration wells.*
3. Micropipettors and tips capable of delivering 50 uL and 100 uL, or disposable pasteur pipets capable of delivering a 50 ± 5 uL drop. Alternatively, an automatic pipettor-dilutor may be used.
4. Glass or plastic test tubes.
5. Stop watch or interval timer.
6. Illuminated white translucent surface.
7. Isotonic saline (0.9% sodium chloride in reagent grade water), commercially prepared isotonic saline without preservative, or phosphate buffered saline; saline pH range should be 6.5 - 7.5.

*It is the user's responsibility to validate an accessory device, whether listed or otherwise, for its intended use. Validation results should be maintained as part of the laboratories records for review by regulatory agencies.

Test Method:

1. Bring reagents to room temperature (18-30 C).

2. Remove Capture-S Microtitration Wells from their protective pouch. If the presence of moisture is shown by the humidity indicator enclosed within each pouch the wells should not be used.
   Note: A batch (or run) is considered to be the largest number of wells used that will be incubated, washed and centrifuged as a unit. Minimum batch size is one strip of wells. The maximum batch size is twelve 1x8 strips fitted into one frame holder. Only one set of controls (one Capture-S Reactive Control well and one Capture-S Nonreactive Control well) is required in each test run.

3. Add 2 drops (100 uL ± 10 uL) of Capture LISS to all test wells.
   Note: The purple color of the Capture LISS will change to a sky or turquoise blue in the presence of serum. The retention of the purple color may indicate that test serum has inadvertently been omitted from the well.

4. Using the vial cap dropper or a pipetting device, add 1 drop (50 ± 5 uL) of Capture-S Reactive Control Serum to the first well.

5. Using the vial cap dropper or a pipetting device, add 1 drop (50 ± 5 uL) of Capture-S Nonreactive Control Serum to the second well.

6. Using a pasteur pipette or micropipettor with disposable tips, add 1 drop (50 uL ± 5 uL) of the first test sample to the third well.

7. Using a clean pipette or new tip, add 1 drop (50 uL ± 5 uL) of the next test sample to the fourth well.

8. Proceed in this manner until all test samples have been added. A clean pipette or new pipette tip must be used for each test sample to prevent cross-contamination.
   Note: Each centrifugation run of test samples must contain at least one set of control reagents (Reactive and Nonreactive Controls) to ensure that the wells have been centrifuged and/or washed properly.

9. Incubate the Capture-S wells at 18-30 C for a minimum of 5 minutes, but no more than 30 minutes.

10. Decant or aspirate the serum-LISS mixture from the wells and wash wells using a manual or automated wash technique.
    a. Manual Washing Technique
       i. Fill the wells with a minimum of 250 uL of saline dispensed from a multichannel dispenser or manifold designed for microtitration plates. Alternatively, a saline wash bottle can be used to dispense the saline.
       ii. Decant the wells thoroughly by manually inverting the strip wells over a waste container and with several rapid, sharp motions, dump the saline from the wells. Personal protective equipment should be used during this procedure.
       iii. Wash the wells six to eight (6-8) times with saline using steps i and ii above. Inspect wells to ensure all fluid has been decanted.
    b. Automated Washing Technique
       i. Prime the instrument and intake lines with isotonic saline according to the instrument manufacturer's directions.
       ii. Wash each well six to eight (6-8) times by filling each well with at least 250 uL of saline and then aspirating the well contents with a vacuum device. Consult the instrument manufacturer's operating manual for a description of the proper use of the microtitration plate washing device. It is recommended, when using an automated microtitration plate washer, to use two 3-cycle or 4-cycle washes. Following the first wash cycle turn the plate 180 degrees. In the event that one of the dispensing or aspirating probes of the washer has become clogged, rotation of the plate increases the likelihood that all test wells will be washed. Alternatively, washers capable of performing a simultaneous dispense and aspiration of saline in the microtitration wells (eg, the CSW 100 washer) may be used. It is recommended that a minimum of 1 mL of saline be used to wash each test well in this simultaneous wash/aspirate mode.

    Note: The automated washing device must be adjusted such that approximately 4-6 uL of saline remains in each well after aspiration. Wells should not be aspirated until they are dry.

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11. Using the vial cap dropper or a pipetting device immediately add 1 drop (50 uL ± 5 uL) of Capture-S Indicator Red Cells to each test well.

12. Centrifuge the wells at 550-850 X g for 2 minutes or a speed and time appropriate for the centrifuge in use. The g force is an approximation of the speed required to produce the required degree of adherence. The appropriate g force (or rpms) and centrifugation time must be determined individually for each centrifuge used.

13. Place the microtitration wells on an illuminated surface and examine for adherence or the absence of Indicator Cell adherence. For test results to be considered valid, the following reactions must be obtained with the Capture-S Control Sera:

    Reactive Control = adherence of Indicator Red Cells over part or all of the reaction surface.
    Nonreactive Control Serum = tight button of Indicator Red Cells at the bottom of the test wells with no area of adherence.

14. Compare each antibody detection test result with those obtained with the reactive and nonreactive control sera. A test should be repeated if a doubtful reaction (irregular, nonconcentric adherence) is obtained or if the control sera do not perform properly.

Stability of Reaction:
Following centrifugation, tests can be read immediately. Since reactive results are permanent, wells can be covered following centrifugation, stored at 1-10 C, and read or reread up to 2 days following testing. ▲

Quality Control:
The reactivity of the Capture-S assay is evaluated at each centrifugation run by inclusion of the provided reactive and nonreactive controls. If, in any test run, the Reactive Control Serum does not produce a reactive result and/or the Nonreactive Control Serum does not produce a nonreactive result, the test run is invalid and all the tests performed in the run must be repeated. Continued failure of the control sera to perform properly may indicate that either one or more of the test reagents has deteriorated, or that the tests are not being performed correctly.

Interpretation of Results:

Nonreactive: Tight button of Capture-S Indicator Red Cells at the bottom of the test well with no area of adherence.

Reactive: Adherence of Capture-S Indicator Red Cells to part of or all of the reaction surface.

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Duplicate repeat testing should be performed on all initially reactive samples. Repeatedly reactive samples should be reported as Reactive. Samples which are nonreactive upon repeat testing should be reported as Nonreactive.

Automated interpretation of Capture-S reactions can be performed utilizing the Inverness Blood Grouping System (IBG) Multireader Plus microplate reader equipped with Screen Test software (IBG Systems Limited; West Sussex, England). The following settings should be incorporated into the Screen Test program:

Valid Range	
Max	Min
8000	2000

Thresholds		
+	?	-
<69	69-70	>70

Reactions interpreted as questionable by the Multireader can be verified by visual examination. Those that are not clearly negative by visual inspection should be retested as described in the paragraphs above. Alternatively, the user can decide, as appropriate, to retest the sample or to exclude the donor unit without retesting or visual editing of reader results.

Limitations:

Test results obtained with Capture-S should be interpreted in conjunction with the donor's history and other clinical and/or laboratory findings. The fluorescent treponemal antibody-absorption test [FTA-ABS] or other recognized treponemal test should be performed on serum samples from the reactive individual.

Biological false-positive reactions have been reported in serological tests for syphilis which utilize cardiolipin type antigens. Acute false-positive reactions which last less than 6 months may occur after viral diseases, febrile diseases, immunizations, or pregnancy.[2] Chronic false-positive reactions may be associated with autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, or leprosy and other conditions such as abnormal immunoglobulin levels and intravenous drug use.[3]

As with all serologic assays, false negative reactions can occur with specimens obtained in the window period between disease onset and seroconversion. False negative reactions due to the prozone reaction in tests utilizing a cardiolipin based antigen have also been reported in the literature.[4,5]

Erroneous test results can occur from bacterial or chemical contamination of test materials, inadequate incubation periods, improper centrifugation, inadequate washing of test wells, or omission of test reagents or steps.
Addition of Capture-S Indicator Red Cells in excess of amounts described in this insert may result in falsely nonreactive or doubtful test reactions.

The Capture-S assay is designed to detect IgG and IgM antibodies. The assay is neither designed to detect antibodies of the IgA or IgE class nor differentiate between IgM and IgG antibodies.

Overcentrifugation of the tests, following addition of the Capture-S Indicator Red Cells, may result in falsely nonreactive or doubtful reactive reactions due to the collapse of the adherent indicator layer. Undercentrifugation will lead to falsely reactive results.

Expected Values:

The incidence and prevalence of syphilis in the donor population can vary geographically and is dependent upon the risk factors associated with the donor population tested. The geographic location and Capture-S initial and repeat reactivity rates for each donor test site are provided in the following table:

Test Site	Geographic Location	Initial Reactive Rate	Repeat Reactive Rate
Site #2	Midwestern United States	1.4%	0.3%
Site #3	Northeastern United States	3.6%	2.8%
Site #4	Southern United States	1.9%	1.9%

Specific Performance Characteristics:

The performance of the Capture-S assay was evaluated at four separate clinical centers on 9085 prospectively collected specimens. The specimen populations tested consisted of serum and plasma samples obtained from blood donors and public health clinic patients. At each center, the specimens were tested by the Capture-S assay and the RPR card test. Confirmatory testing using the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test was performed on all samples reactive in the Capture-S and/or RPR card test. The results of these evaluations are summarized in the following table.

Consensus Results from Clinical Trial Sites

	Capture-S	RPR Card Test
Sensitivity	94.0 % (267/284)	91.2 % (239/262)
Specificity	99.2 % (8686/8759)	99.3% (6120/6166)

There was 98.1 % (8915/9085) agreement between the Capture-S assay results and the results obtained with the RPR card test at these sites. Of the 170 samples with differing reactivities between the two assays, 121 samples were reactive in the Capture-S assay but nonreactive in the RPR card test, while 49 samples were reactive in the RPR card test but nonreactive with Capture-S. The sensitivity and specificity of the Capture-S assay with the calculated 95% confidence intervals at each donor test site and a clinical syphilis site (Site #5) are summarized in the following table.

Test Site	Sensitivity		Specificity	
	Value	†95% CI	Value	95% CI
Site #1	94.6%	91.7-97.5	98.7%	98.47-98.93
Site #2	100%	50.0-100	99.3%	99.26-99.33
Site #3	93.8%	83.8-100	98.6%	98.53-98.68
Site #4	83.3%	57.1-100	99.9%	99.88-99.93
Site #5	80.2%	78.0-82.4	96.2%	95.09-97.31

†95% CI = 95% Confidence Interval

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The clinical sensitivity 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, or an unknown stage of the disease. A summary of the comparative study are presented in the following table.

Clinical Sensitivity

Syphilis Category	n	Capture-S (R)		Capture-S (N)		Sensitivity	
		RPR (R)	RPR (N)	RPR (R)	RPR (N)	Capture-S	RPR
Primary							
Untreated	29	23	0	2	4	88.5 %	96.2 %
Treated	54	18	14	3	19	62.5 %	43.8 %
Secondary							
Untreated	50	45	0	3	1	91.8 %	97.9 %
Treated	89	66	5	8	10	80.2 %	82.6 %
Latent							
Untreated	41	37	2	0	2	94.9 %	92.3 %
Treated	80	53	6	8	13	75.0 %	80.3 %
Unknown							
Untreated	12	9	1	0	2	83.3 %	75.0 %
Treated	11	8	1	0	2	81.8 %	72.7 %
Total	**366**	**259**	**29**	**24**	**53**	**80.7%**	**80.3%**

(R) = Reactive (N) = Nonreactive

Reproducibility:

The reproducibility of the Capture-S assay was evaluated at each test site on identical panels of sixty (60) samples consisting of ten (10) samples of each of six serum pools. The Capture-S assay demonstrated 100% reproducibility at each test site and, thus, a 100% agreement (240 of 240) between test sites. The RPR card test demonstrated a 96.3% agreement (231 of 240 samples) of reproducibility panel test results between the test sites. The Capture-S assay demonstrated a 100% within-day and 100% day-to-day reproducibility.

The IBG Multireader Plus, at thresholds of <69/>70 demonstrates 98.34% concordance with all visual Capture-S results. Percent concordance of Multireader interpretations with each type of result (negative, positive and questionable) is shown below.

Visually positive reactions interpreted by reader as:	
Positive	168 (93.33%)
Questionable	12 (6.67%)
Negative	0 (0.00%)
Total	180
Visually negative reactions interpreted by reader as:	
Negative	3301 (98.98%)
Questionable	33 (0.98%)
Positive	1 (0.03%)
Total	3335
Visually questionable reactions interpreted by reader as:	
Questionable	81 (86.17%)
Positive	12 (12.77%)
Negative	1 (1.06%)
Total	94

Clinical testing to determine 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 and RPR card test were used to test specimens from the following categories: Systemic Lupus Erythematosis and/or anti-nuclear antibody (ANA; anti-DNA)-positive, drug addicts, post-Hepatitis B virus vaccination (anti-HBs), Legionella-positive, Lyme disease, infectious mononucleosis, Mycoplasma-positive, multiple myeloma, pregnancy, Rheumatoid Factor (RF)-positive, and Rubella-positive specimens. The results of the clinical specificity testing are presented in the table below:

Clinical Specificity Testing

Sample Category	n	Capture-S (R)		Capture-S (N)	
		RPR (R)	RPR (N)	RPR (R)	RPR (N)
ANA(+); anti-DNA(+)	10				10
Drug Addicts	10		1(a)		9
anti-HBs(+)	10				10
Legionella	10			1(b)	9
Lyme Disease	10				10
Mononucleosis(+)	10				10
Mycoplasma(+)	10	1(a)		1(b)	8
Multiple Myeloma	10				10
Pregnancy	10				10
RF(+)	10			1(b)	9
Rubella	10				10
TOTAL	110	1	1	3	105

(R) = Reactive (a) = MHA-TP Reactive
(N) = Nonreactive (b) = MHA-TP Nonreactive

▲ The performance of this product is dependent upon adhering to the insert's recommended methodology. Additional information regarding testing performed at the time of manufacture may be furnished upon request by consulting Immucor's Technical Service at 800-492-BLUD (2583) or 770-441-2051.

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1. Cannefax GR, Norins LC, and Gillespie EJ. Immunology of syphilis. Annu Rev Med 1967;18:471-82.
2. Larsen SA, Hunter EF, Kraus SJ, eds. Diagnostic tests, In: A Manual of Tests for Syphilis. Washington: American Public Health Association, 1990:1-26.
3. Holmes KK, Lukehart SA. Syphilis, In: Harrison's Principles of Internal Medicine, 11th Ed. Braunwald E, et al. New York: McGraw Hill, 1987: Ch.122.
4. Spangler AS, Jackson J, Fiumara NJ, Warthin TA. Syphilis with a negative blood test reaction. JAMA 1964; 189:113-116.
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