CAPTIA™ Syphilis (T. Pallidum)-G

INTENDED USE

CAPTIA™ Syphilis (T. pallidum)-G is an enzyme immunoassay for the qualitative detection of IgG antibodies to T. pallidum in serum specimens, to be used in conjunction with non-treponemal testing to provide serological evidence of infection with T. pallidum (the agent of syphilis).

CAPTIA™ Syphilis (T. pallidum)-G is also intended for testing of serum or plasma specimens to screen blood and/or plasma donors to exclude a history of syphilis.

For in vitro Diagnostic Use Only

For Professional Use Only

Warning: A positive result is not useful for establishing a diagnosis of syphilis. In most situations, such a result may reflect a prior treated infection; a negative result can exclude a diagnosis of syphilis except for incubating or early primary disease.

INTRODUCTION

Syphilis is a disease, usually sexually transmitted, caused by infection with the spirochete Treponema pallidum (T. pallidum). Infection is systemic from the outset and the disease is characterized by periods of latency, often in excess of twenty years. These features, together with the fact that T. pallidum cannot be isolated in culture mean that serological techniques play a major role in the diagnosis of syphilis and treatment follow-up.1

The procedures most commonly used to screen for antibodies to T. pallidum in clinical diagnostic laboratories are based upon their reaction with non-treponemal lipoidal antigens (the reagin tests). Reagin tests, such as the RPR or VDRL, can be used to test serial dilutions of the serum specimen. The end point values from sequentially obtained serum samples decline following successful treatment until after a period of several months the patient will usually become reagin test non-reactive.

Clinical diagnostic serum specimens which are reactive in reagin tests are typically confirmed using treponemal tests such as the Microhaemaggulination-T. pallidum (MHA-TP) or the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) test. In contrast to the non-treponemal tests, treponemal T. pallidum treatment until after a period of several months the patient will usually become reagin test non-reactive. Indications of deterioration:

1. The kit fails to meet the required criteria for a valid test (see INTERPRETATION).
2. Reagents becoming visibly cloudy or develop precipitate. Note: Concentrated wash buffer, when cold, normally develops crystalline precipitates which redissolve on heating at 37 C.
3. The CAPTIA™ Substrate Solution is blue. This is likely to be caused by chemical contamination of the CAPTIA™ Substrate Solution or the container.

Please note Substrate Solution when supplied has a slight blue coloration.

SAFETY

For in vitro Diagnostic Use Only. For Professional Use Only

The control sera in this kit contain < 0.1% sodium azide as preservative. Sodium azide can react with lead and copper plumbing to form potentially explosive metal azides. Upon disposal, 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 kit control sera were derived was found negative when tested in accordance with current FDA recommendations. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.

The Treponema pallidum antigen has been inactivated during the production processes. Nevertheless, treponemal antigen-coated microtiter wells should be handled using the normal precautions accorded to potentially infectious material.

Sulfuric acid is corrosive. Avoid contact with skin and eyes. If splashing onto skin or eyes occurs, rinse the affected area with copious quantities of water and seek medical attention.
PROcedural

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. CAPTIA™ enzyme immunoassay kits contain reagent systems which are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.
3. Do not use CAPTIA™ Syphils-G kits after the expiration date printed on the outer carton label.
4. Do not cross-contaminate reagents. Always use fresh pipette tips when drawing from stock reagent bottles.
5. Always use clean, preferably disposable, glassware for all reagent preparation.
6. Allow reagents to warm to room temperature before opening. This avoids condensation on the inner surface of the bag which may contribute to a deterioration of coated strips intended for future use.
7. Reagents should be dispensed with the tip of the micropipette touching the side of the well at a point about mid-section. Follow manufacturer’s recommendations for automatic processors.
8. Always keep the upper surface of the microtiteration strips free from excess fluid droplets. Reagents and buffer overspill should be blotted dry on completion of the manipulation.
9. Do not allow the wells to completely dry out during an assay.
10. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor. Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910.1030) FEDERAL REGISTER, pp 64:176-84:177; 12/29/91.
11. Automatic or semi-automatic EIA processors or liquid handling systems should be qualified specifically for use with CAPTIA™ Syphils-G by demonstration of equivalence to the manual processing methods.
12. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic) are regularly calibrated according to the manufacturer’s instructions.
13. Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

METHOD OF USE

SPECIMEN COLLECTION AND STORAGE

CAPTIA™ Syphils-G is intended for use with serum, EDTA or citrated plasma samples. If serum specimens are to be stored, they may be stored at 2-8°C for up to five days. However, if storage periods greater than five days are anticipated, the serum specimens should be stored frozen at -20°C or below. Specimens which have been frozen and thawed should be thoroughly mixed before testing. If plasma specimens are to be tested, they may be stored at 2-8°C for up to 48 hours. Plasma specimens should not be frozen due to fibrin clot formation.

Optimal performance of the Trinity Biotech ELISA kit depends upon the use of fresh serum/plasma samples (clear, non-hemolyzed, non-lipemic, non-icteric).

If serum specimens are to be shipped, they should be packaged and labeled in compliance with federal and international regulations covering the transportation of clinical specimens and etiologic agents. Serum specimens may be shipped ambient, refrigerated (2-8°C) or on wet ice or frozen (-10°C or colder) on dry ice. Upon arrival, if serum specimens are to be stored, they may be stored at 2-8°C for up to five days after collection or frozen (-20°C or colder). The NCCLS provides recommendations for storing blood specimens (Approved Standard – Procedures for the Handling and Processing of Blood Specimens, H 18-A. 1990).

RINSE CYCLE

Efficient rinsing to remove uncomplexed sample components is a fundamental requirement of enzyme immunoassay procedures. CAPTIA™ Syphils-G utilizes two standard five-rinse cycles. Automatic plate washers may be used providing they meet the following criteria:

• All wells are completely aspirated.
• 2. All wells are filled to the rims (350 μL) during the rinse cycle.
• 3. Wash buffer is dispensed at a good flow rate.
• 4. The microtiteration plate washer must be well maintained to prevent contamination from previous use. Manufacturer’s cleaning procedures must be followed diligently.
• 5. Validated by end user

For each rinse cycle, the machine should be set to five consecutive washes. On completion of the cycle, invert the microtiteration plate and tap firmly on absorbent paper towels. Check for any residual wash buffer in the wells and blot dry the upper surface of the wells with a paper towel. Alternatively, the following manual system may be employed:

1. Aspirate well contents using a vacuum line fitted with a trap.
2. Fill all wells to the brim with wash buffer dispensed from a squeeze-type laboratory wash bottle.
3. Aspirate all wells.
4. Repeat Steps 2 and 3, four times.
5. Insert the microtiteration plate and tap firmly on absorbent paper towels.

PREPARATION FOR THE ASSAY

Specimen Dilution

Test specimens should be tested following a 1:21 dilution with CAPTIA™ Sample Diluent. This dilution can be performed off-plate in tubes, or directly in the microtiter well. Tube dilution is recommended for diagnostic lab use. In-well dilution is recommended for high volume (blood banks) screening.

Tube method

This is most accurately performed in disposable tubes of 1.0 - 1.5 mL capacity, which can be arranged in 8 x 12 - place racks that conform to microtiteration plate geometry. This facilitates later transfer of diluted specimens from the tubes to the test microtiteration plate using a multichannel pipette.

Dispense 1.0 mL of CAPTIA™ Sample Diluent into each tube. Add 50 μL of specimen and mix 5 to 8 times. Use a fresh disposable pipette tip for each serum specimen.

In-well method

Dispense 200 μL of CAPTIA™ Sample Diluent into all the wells designated for test specimens. Dispense 10 μL of each specimen into its designated well. Pump mixture in and out of the microtitration plate in order to ensure full delivery. When dispensing of test specimens into the microtiteration plate is complete and the working strength controls have also been dispensed, mix the well contents thoroughly by shaking on a mechanical microtiteration plate shaker for 5-10 seconds, or by mixing in-probe if automatic EIA processors are used. Thorough mixing is essential.

Kit Controls

Note: The kit controls provided are working strength and do not require dilution.

1. Allow all reagents to reach room temperature (18-25°C).
2. The kit controls should be included on each microtiteration plate. The Low Titre Reactive Control should always be tested in duplicate. Additionally, it is recommended that a well characterized low titre reactive specimen, or an independent reference sample, diluted in Sample Diluent, be included in each microplate set-up.

Wash Buffer

The 20 x concentrated CAPTIA™ Wash Buffer may have developed crystalline deposits during prolonged storage at 2-8°C. These should be redissolved by standing the bottle in a 37°C water bath until the crystals disappear. Prepare working-strength Wash Buffer by diluting 1 part concentrate with 19 parts of distilled or deionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs (see STORAGE AND STABILITY). Each row of 8 wells may be adequately washed with 150 μL of working strength buffer.

ASSAY PROCEDURE

1. Allow all reagents to reach room temperature (18-25°C).
2. The kit controls should be included on each microtiteration plate. The Low Titre Reactive Control should always be tested in duplicate.
3. Select sufficient microtiteration plate strips to accommodate test specimens and controls. Fit the strips into the holding frame. Label wells according to specimen: identify using the letter/number cross-reference system molded into the plastic.
4. If the tube dilution method has been used, dispense 100 μL of each diluted serum or plasma specimen into the correspondingly pre-labeled wells. Mix the diluted specimen 6 to 8 times using the 100 μL micropipette, prior to transfer. Use a fresh micropipette tip for each diluted specimen.
5. Seal the strips and holding frame with a plate sealer and incubate at 37(± 1) C for 60 (±5) minutes.
6. Aspirate diluted specimen from the wells and wash the microtiteration plate as described in the Rinse Cycle section.
7. Dispense 100 μL conjugate into each well, seal the strips with a plate sealer and incubate at 37(± 1) C for 60 (± 5) minutes.
8. Aspirate the conjugate from the wells and wash the microtiteration plate as described in the Rinse Cycle section.
9. Without delay, dispense 100 μL of substrate solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25°C) protected from direct sunlight, for 30 (±5) minutes.
10. Stop the reaction by adding 100 μL of stop solution (1 N sulfuric acid) to each well. Mix on a plate shaker for 5 to 10 seconds, or tap lightly. This is to ensure that the blue solution changes to a uniform yellow color in each well. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.
11. Within 30 minutes of adding the acid, read the absorbance values at 450 nm using a microtiteration plate reader blanked on air unless the manufacturer specifically recommends otherwise.

INTERPRETATION

ASSAY VALIDATION

A CAPTIA™ Syphils-G assay should be considered valid if:

• The absorbance of the Non-reactive Control (N) is less than or equal to 0.25.
• The absorbance value of the High Titre Reactive Control is greater than or equal to 0.8.
• The mean absorbance value of the Low Titre Reactive Control (LTR) is greater than or equal to 2.5 x the absorbance of the Non-reactive Control.

TROUBLESHOOTING ADVICE

If the kit controls fail to give the absorbance values indicated above, the following points should be considered:

1. Ensure that the details given in Procedural have been reviewed.
2. For a High Absorbance Non-reactive Control (>0.25):
   • Ensure that the washing procedure detailed in Methods for Use: Rinse Cycle was followed. If using an automatic washer, ensure all inlet and outlet probes are not blocked and that all wells are being illed and aspirated fully.
3. For a Low Absorbance High Titre Reactive Control (<=0.8):
   • Check that the correct incubation conditions (time and temperature) were achieved.
   • Ensure that all residual wash buffer has been removed from the wells after use.
   • For a LTR ratio <2.5, check all points detailed in (1) to (3) of this section.
   • After consideration of the points above, repeat the assay. If the kit controls again fail to validate, contact your supplier for further advice.

ANALYSIS
Calculate the mean absorbance value of the duplicate Low Titre Reactive Controls. This is the cut-off value for CAPTIA™ Syphilis-G and was derived from clinical trials as the value giving optimum discrimination between specimens which are reactive or non-reactive for antibodies to T. pallidum as characterized by a range of standard serological techniques.

Specimen absorbance values within 10% of the mean of the Low Titre Reactive Controls should be considered equivocal results.

A specimen may be considered reactive for IgG antibodies to T. pallidum if it gives an absorbance value greater than the mean of the Low Titre Reactive Controls and outside the equivocal range.

It is often useful to ascribe a numerical value to a specimen which represents its CAPTIA™ Syphilis-G reactivity so that comparisons can be made between different assays. Such a value is derived by expressing the absorbance of the test specimen as a ratio of the mean absorbance of the kit Low Titre Reactive Controls.

For example:

- Test serum absorbance = 0.75
- Mean LTR* absorbance = 0.30
- Antibody Index = 0.75 / 0.30 = 2.50

*LTR - Low Titre Reactive Control

An Antibody Index between 0.9 and 1.1 should be considered equivocal.

An Antibody Index greater than or equal to 1.1 is a reactive result and an Index less than or equal to 0.9 is a non-reactive result.

A CAPTIA™ Syphilis-G reactive result (following a reactive nontreponemal test result in the diagnostic application) indicates a current or past infection with T. pallidum.

A non-reactive result indicates absence of infection at any time more than 2-3 weeks previous to drawing the specimen. (See Limitations of Use).

CAPTIA™ SYPhILIS-G USED AS A CLINICAL LAB TEST.

Initially reactive or equivocal results should be repeated in duplicate. If the repeat test is again equivocal a fresh serum specimen should be tested. The CAPTIA™ Syphilis-G is a treponemal assay, therefore patients with previously treated syphils will be positive on the assay. The test can not distinguish between present and past infection. Any sera giving reactive or equivocal results on initial testing must be supplemented with a quantitative nontreponemal test (such as RPR and VDRL) to distinguish active disease and assist in ruling out false positives.

The following table is used for result interpretation:

<table>
<thead>
<tr>
<th>Non-Treponemal Result (NT)</th>
<th>Treponemal Result</th>
<th>Report/Interpretation for all except neonates or infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>nonreactive (negative)</td>
<td>negative (nonreactive)</td>
<td>No serological evidence of infection with T. Pallidum (incubating or early primary syphilis cannot be excluded).</td>
</tr>
<tr>
<td>reactive</td>
<td>reactive (reactive)</td>
<td>Current infection unlikely, probability of biological false-positive secondary to other medical conditions (folic acid deficiency, immunizations, IV drug, autoimmune diseases, etc.). Recommend repeat testing (nontreponemal and treponemal by other test method).</td>
</tr>
<tr>
<td>nonreactive (positive)</td>
<td>reactive (reactive)</td>
<td>Probably past infection or potential cross-reactivity with other spirochetes/related antigens; additional testing appropriate to clinical findings/history; possibility of false negative nontreponemal (NT) due to prozone and late syphilis or neurosyphilis.</td>
</tr>
<tr>
<td>reactive</td>
<td>reactive (reactive)</td>
<td>Presumptive evidence of current infection (or inadequately treated infection, persistent infection, re-infection, or biological false positive if prior history); additional testing consistent with clinical assessment.</td>
</tr>
<tr>
<td>nonreactive (negative)</td>
<td>not done (negative)</td>
<td>Current infection unlikely; effectively treated infection if previous diagnosis and treatment; cannot exclude incubating or early primary syphilis; cannot exclude latent or neurosyphilis.</td>
</tr>
<tr>
<td>not done</td>
<td>not done (negative)</td>
<td>Current or past infection unlikely; cannot exclude incubating or early syphilis.</td>
</tr>
</tbody>
</table>

1. Quantitative nontreponemal testing; clinical history; repeated (sequential) serological testing for changes in titer.

HIV-infected individuals may have delayed seroconversion or negative serology.

CAPTIA™ SYPhILIS-G USED AS A PRIMARY BLOOD OR PLASMA DONOR SCREEN FOR BLOOD BANKS AND PLASMAPHARESIS CENTERS.

Initially reactive or equivocal results should be repeat tested in duplicate. Where plasma specimens have been tested initially, serum specimens are preferred for retesting. If the repeat test is again reactive or equivocal the specimen should be referred for confirmation to a Syphilis Reference Laboratory. Specimens which give repeat equivocal results by CAPTIA™ should be considered reactive until confirmatory testing (RPR, VDRL) has been done. In practice, equivocal results are found in less than 0.5% of specimens tested (see Trial 9 in Performance Characteristics).

CAPTIA™ SYPhILIS-G USED AS A DIAGNOSTIC CONFIRMATORY TEST

Specimens giving equivocal results should be repeated in duplicate. If the result is again equivocal, a fresh serum specimen should be tested. When retesting sera giving equivocal results on initial testing, the sample should be tested using a quantitative nontreponemal test and another treponemal test. A second serum sample should also be obtained (one drawn at a later date) if results of the nontreponemal and treponemal tests do not resolve the diagnosis.

EXPECTED VALUES

The percentage of specimens reactive by CAPTIA™ Syphilis-G is dependent upon the population from which the specimens were derived. A can be expected that specimens derived from ‘high risk’ patients (e.g. those attending genitourinary clinics) will show a higher reactivity rate than those derived from a low risk population (e.g. blood donors). Also the number of specimens reactive will be dependent upon the type of laboratory carrying out the testing - a Reference Laboratory testing samples that may already have been screened for syphilis by a serological assay will have a higher incidence of reactive specimens than a routine Clinical Laboratory testing specimens for the first time.

From the results presented in the section Performance Characteristics it could be expected that a Clinical Laboratory testing referred specimens and routine specimens including those from genitourinary clinics may have a reactivity rate of approx. 4.5% (61/1321 - Trial 1).

From the data presented in Trial 9, it could be expected that a Blood Testing Center would obtain approx. 0.8% (73/9323) reactive specimens using CAPTIA™ Syphilis G.

LIMITATIONS OF USE

1. Results from CAPTIA™ Syphilis-G should be considered in the context of all available clinical and laboratory data.
2. A CAPTIA™ Syphilis-G non-reactive result does not preclude the possibility of:
   • a very recent infection (within the last 2-3 weeks) with T. pallidum.
   • an old, successfully cured infection with T. pallidum (for example >10 years previous).
3. CAPTIA™ Syphilis-G may be reactive with sera from patients with Yaws (T. pallidum subspecies pertenue) or Perico (T. carateum).
4. Detection of treponemal antibodies may indicate recent, past, or successfully treated syphilis infections, therefore, the test cannot be used to differentiate between active and cured cases.
5. When performing clinical lab assays, any sera giving reactive or equivocal results must be supplemented with a quantitative nontreponemal test (such as RPR and VDRL) to distinguish active disease and assist in ruling out false positives. The CAPTIA™ Syphilis-G is a treponemal assay, therefore patients with previously treated syphils will be positive on the assay.
6. The use of CAPTIA™ Syphilis-G as an initial screening test for blood donors may result in higher numbers of reactive donors who may not be currently infected compared to screening donors with standard, nontreponemal assays. It is recommended that CAPTIA™ Syphilis-G repeat reactive specimens be tested by methods capable of indicating the current disease status of the donor e.g. RPR and VDRL tests.
7. AIDS patients with impaired immunity and who are co-infected with syphilis may react falsely nonreactive in treponemal and nontreponemal tests.
8. Reactive treponemal IgG antibody test results usually remain reactive for a lifetime, therefore antibody indices cannot be used to determine response to therapy.

PERFORMANCE CHARACTERISTICS

CLINICAL LAB TEST APPLICATION

Comparison with other serological tests.

CAPTIA™ Syphilis-G has been evaluated at a number of independent clinical laboratories. Serum specimens routinely referred for syphilis serology were analyzed by CAPTIA™ Syphilis-G in parallel with haemagglutination (MHA-TP) and VDRL tests. Specimens giving reactive results by any test were further tested by the FTA-ABS procedure. The tables below summarize results from initial testing at two trial centers. In all cases a reactive is defined as a serum specimen which gives a reactive result by either the MHA-TP or VDRL test and which is confirmed by the FTA-ABS test.

Trial 1

<table>
<thead>
<tr>
<th>Total 1321 Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. pallidum antibody status</td>
</tr>
<tr>
<td>Reactive</td>
</tr>
<tr>
<td>Non-reactive</td>
</tr>
<tr>
<td>N = Non-reactive</td>
</tr>
</tbody>
</table>

Note: Equivalors scored as reactive:

* Confirmed untreated primary infection, reactive by CAPTIA™ Syphilis-G.

† Includes 7 specimens in equivocal range.

‡ On repeat testing confirmed as non-specific agglutination, therefore are inconclusive.

§ Weakly reactive case of treated latent syphilis.

∥ The proportion of this group representing cases of successfully treated syphilis, which would not normally be VDRL reactive, is not known.
The remaining 516 serum specimens were non-reactive in both the RPR test and Of the 585 specimens tested, only 69 were initially reactive, and then tested in the FTA-ABS procedure. The following table illustrates the performance of the assay with a characterized serum panel and does not infer an endorsement of the assay by the CDC.

### Results of the CDC Serum Panel on the Captia Syphilis G

<table>
<thead>
<tr>
<th>Stage</th>
<th>RPR Reactive</th>
<th>CAPTIA™ Syphilis-G Reactive</th>
<th>FTA-ABS Reactive</th>
<th>Total</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Treated</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>16</td>
<td>53.8%</td>
</tr>
<tr>
<td>Primary Untreated</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>100%</td>
</tr>
<tr>
<td>Secondary Treated</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>100%</td>
</tr>
<tr>
<td>Secondary Untreated</td>
<td>21</td>
<td>1</td>
<td>22</td>
<td>95.5%</td>
<td></td>
</tr>
<tr>
<td>Late Treated</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>21</td>
<td>95.2%</td>
</tr>
<tr>
<td>Late Untreated</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>12</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>0</td>
<td>3</td>
<td>100</td>
<td>97%</td>
</tr>
</tbody>
</table>

### Results of the characterized Serum Panel on the Captia Syphilis G

<table>
<thead>
<tr>
<th>Disease</th>
<th>RPR Reactive</th>
<th>CAPTIA™ Syphilis-G Reactive</th>
<th>FTA-ABS Reactive</th>
<th>Total</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Treated</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>Primary Untreated</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>20</td>
<td>95%</td>
</tr>
<tr>
<td>Secondary Treated</td>
<td>27</td>
<td>1</td>
<td>1</td>
<td>29</td>
<td>93.1%</td>
</tr>
<tr>
<td>Secondary Untreated</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>100%</td>
</tr>
<tr>
<td>Late Treated</td>
<td>25</td>
<td>0</td>
<td>1</td>
<td>26</td>
<td>96.1%</td>
</tr>
<tr>
<td>Late Untreated</td>
<td>92</td>
<td>1</td>
<td>0</td>
<td>93</td>
<td>98.9%</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>3</td>
<td>2</td>
<td>200</td>
<td>97.5%</td>
</tr>
</tbody>
</table>

Provided below is a Summary Table of Trials 3, 4, 6 and 7 of data obtained from collections which were characterized by syphilis disease states:

### Summary Table from Trials 3, 4, 6, 7

<table>
<thead>
<tr>
<th>Patient Category</th>
<th># Patients</th>
<th>RPR/VORL Results</th>
<th>CAPTIA™ Syphilis-G Results</th>
<th>CAPTIA™ Syphilis-G Reactive or FTA or VDRL Reactive</th>
<th>RPR or VDRL Reactive and FTA Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Syphilis</td>
<td>50</td>
<td>46</td>
<td>46</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Secondary</td>
<td>60</td>
<td>60</td>
<td>59</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Latent</td>
<td>117</td>
<td>115</td>
<td>216</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Treated Syphilis</td>
<td>26</td>
<td>26</td>
<td>25</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Secondary</td>
<td>59</td>
<td>59</td>
<td>57</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Latent</td>
<td>62</td>
<td>60</td>
<td>60</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Not: Equivocal scored as reactive.

* Includes 2 serum specimens which, on repeat testing, were confirmed as causing non-specific agglutination, therefore are inconclusive.

* The proportion of this specimen group representing cases of successfully treated syphilis, which would not normally be VDRL reactive, is not known.
Trial 8

RPR Positive and FTA Negative Sera
Two sites (Public Health Labs located in New York and Maryland) tested 25 frozen retrospective sera on the CAPTIA™ Syphilis G that were RPR positive on initial screen and FTA negative on confirmation. The following table summarizes the results.

<table>
<thead>
<tr>
<th>CAPTIA™ Syphilis Positive</th>
<th>CAPTIA™ Syphilis Equivocal</th>
<th>CAPTIA™ Syphilis Negative</th>
<th>% Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Site 2</td>
<td>0</td>
<td>2*</td>
<td>23</td>
</tr>
</tbody>
</table>

*The two equivocals were negative with repeat testing.

Trial 9

Blood and Plasma Donor Screening/Application

Comparison with the RPR test

CAPTIA™ Syphilis-G was evaluated at 2 major US blood centers and a plasma center, in comparison with the RPR test. A total of 9,323 donors were tested as plasma specimens by CAPTIA™ Syphilis-G and as serum specimens by the RPR test. 152 of the specimens were known reactive from previous serological testing which did not include the FTA-ABS test. 4,274 of these donors were additionally tested as serum specimens using CAPTIA™ Syphilis-G. Initially-reactive specimens were repeat tested in duplicate. Repeat-reactive specimens were confirmed using an FTA-ABS test. The following tables compare the CAPTIA™ Syphilis-G and RPR before and after reconciliation of discordant results by the FTA test.

CAPTIA™ Tests Plasma Specimens: CAPTIA™ Syphilis-G, RPR and FTA-ABS Results

<table>
<thead>
<tr>
<th>CAPTIA™ Syphilis-G (Tests Plasma Specimens)</th>
<th>RPR</th>
<th>Reactive and Discordants Reconciled by FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR NUMBER FTA-ABS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>123</td>
<td>0</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>967</td>
<td>0</td>
</tr>
<tr>
<td>Relative Specificity</td>
<td>967</td>
<td>99.75%</td>
</tr>
<tr>
<td>Relative Sensitivity</td>
<td>967</td>
<td>99.75%</td>
</tr>
<tr>
<td>% Agreement</td>
<td>967</td>
<td>99.75%</td>
</tr>
</tbody>
</table>

Comparison with an automated MHA-TP (haemagglutination) test

CAPTIA™ Syphilis-G was evaluated at two major US blood centers, in comparison with a commercially available automated MHA-TP system (PK-TP test). A total of 6,196 donors were tested as plasma specimens by CAPTIA™ Syphilis-G. 2,156 of these donors were additionally tested as serum specimens by CAPTIA™ Syphilis-G. The MHA-TP initial tests were performed with plasma specimens, and MHA-TP repeat tests were performed with serum specimens. Specimens which were repeat reactive in either test were confirmed using an FTA-ABS test.

The following tables summarize the results of all tests and compare CAPTIA™ and MHA-TP before and after reconciliation of discordant results by the FTA-ABS tests.

CAPTIA™ Tests Plasma Samples: CAPTIA™ Syphilis-G, MHA-TP and FTA-ABS Results

<table>
<thead>
<tr>
<th>CAPTIA™</th>
<th>MHA-TP</th>
<th>NUMBER</th>
<th>FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR</td>
<td>Reactive</td>
<td>Non-reactive</td>
<td>FTA-ABS</td>
</tr>
<tr>
<td>R</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>N</td>
<td>86</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>% Agreement</td>
<td>99.75%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Relative Sensitivity</td>
<td>99.75%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Relative Specificity</td>
<td>99.80%</td>
<td>99.80%</td>
<td></td>
</tr>
</tbody>
</table>

Of 124 samples that were repeat reactive in either or both tests (CAPTIA™ and MHA-TP), 105, were FTA-ABS reactive and 19 were FTA-ABS non-reactive.

CAPTIA™ Tests Serum Samples: CAPTIA™ Syphilis-G, MHA-TP and FTA-ABS Results

<table>
<thead>
<tr>
<th>CAPTIA™</th>
<th>MHA-TP</th>
<th>NUMBER</th>
<th>FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR</td>
<td>Reactive</td>
<td>Non-reactive</td>
<td>FTA-ABS</td>
</tr>
<tr>
<td>R</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>N</td>
<td>86</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>% Agreement</td>
<td>99.75%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Relative Sensitivity</td>
<td>99.75%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Relative Specificity</td>
<td>99.80%</td>
<td>99.80%</td>
<td></td>
</tr>
</tbody>
</table>

* includes 6072 specimens which were CAPTIA™ and MHA-TP non-reactive and therefore not tested by FTA-ABS

4/5 FTA Non-Reactives

Equivocals were scored as reactive. In this study there were 18 specimens (0.23%) initially equivocal by CAPTIA™, and 9 specimens (0.15%) repeat equivocal.

CAPTIA™ Tests Serum Specimens: CAPTIA™ Syphilis-G, RPR and FTA-ABS Results

<table>
<thead>
<tr>
<th>CAPTIA™</th>
<th>RPR</th>
<th>NUMBER</th>
<th>FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>Non-reactive</td>
<td>FTA-ABS</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>N</td>
<td>86</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>% Agreement</td>
<td>99.75%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Relative Sensitivity</td>
<td>99.75%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Relative Specificity</td>
<td>99.80%</td>
<td>99.80%</td>
<td></td>
</tr>
</tbody>
</table>

Of 98 samples that were repeat reactive in either or both tests (CAPTIA™ and MHA-TP), 95 were FTA-ABS reactive, and three were FTA-ABS non-reactive.

CAPTIA™ (Tests Plasma Specimens) | MHA-TP | Reactive and Discordants Reconciled by FTA-ABS |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR NUMBER FTA-ABS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>% Agreement</td>
<td>99.75%</td>
<td>-</td>
</tr>
<tr>
<td>Relative Sensitivity</td>
<td>99.75%</td>
<td>-</td>
</tr>
<tr>
<td>Relative Specificity</td>
<td>99.80%</td>
<td>99.80%</td>
</tr>
</tbody>
</table>

* includes 2058 specimens which were CAPTIA™ and MHA-TP non-reactive and therefore not tested by FTA-ABS

a 4/5 FTA Non-Reactives

Equivocals were scored as reactive. In this study there were 10 specimens (0.46%) initially equivocal by CAPTIA™, and 9 specimens (0.42%) repeat equivocal.

CAPTIA™ (Tests Plasma Specimens) | MHA-TP | Reactive and Discordants Reconciled by FTA-ABS |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR NUMBER FTA-ABS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>% Agreement</td>
<td>99.75%</td>
<td>-</td>
</tr>
<tr>
<td>Relative Sensitivity</td>
<td>99.75%</td>
<td>-</td>
</tr>
<tr>
<td>Relative Specificity</td>
<td>99.80%</td>
<td>99.80%</td>
</tr>
</tbody>
</table>

Of 124 samples that were repeat reactive in either or both tests (CAPTIA™ and MHA-TP), 105, were FTA-ABS reactive and 19 were FTA-ABS non-reactive.
CAPTIA™ Syphilis-G: comparison of performance with plasma and serum specimens

A total of 4,274 serum/plasma pairs were tested at 2 major US blood centers and a plasma center using CAPTIA™ Syphilis-G. Results are summarized in the following table. 4,258 pairs (95.62%) gave concordant results on initial testing (equivocal scored as positive). Eleven (11) specimen pairs gave discordant results on repeat testing. Of these 11 repeat discordant pairs, the FTA-ABS test confirmed the plasma result for 6 pairs, and the serum results for the remaining 5 pairs.

The reproducibility of CAPTIA™ Syphilis-G was evaluated concurrently at 3 separate US blood/plasma centres. Each centre tested 6 standard serum samples, replicated x 3 in each assay, on each of 5 days. The serum samples comprised: 2 x high titre reactive serum specimens; 2 x low titre (near the cut-off) reactive serum specimens and 2 x non-reactive serum specimens. Results are summarized in the following table:

### SPECIFICITY AND CROSS-REACTIVITY

The following table summarizes CAPTIA™ Syphilis-G results from serum specimens taken from subjects with known history or serological evidence of syphilis. This group included serum specimens representing other disease states and/or characteristics known to cause false reactives in serum specimens and 2 x non-reactive serum specimens. Results are summarized in the following table:

### REPRODUCIBILITY

The reproducibility of CAPTIA™ Syphilis-G was evaluated at two separate Public Health Labs. Each centre tested 6 standard serum samples, replicated x 3 in each assay, on each of 5 consecutive days. The same 6 samples were then replicated x 3 in each assay, separated by one week intervals for five weeks. The last two assays were each performed with different lots of kits. The serum samples comprised: 2 x high titre reactive serum specimens; 2 x low titre (near the cut-off) reactive serum specimens and 2 x non-reactive serum specimens. Results are summarized in the following table:

---

**CAPTIA™ Syphilis-G: comparison of performance with plasma and serum specimens**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Plasma</th>
<th>Initial</th>
<th>Repeat</th>
<th>Reactive</th>
<th>FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>Equivocal</td>
<td>154</td>
<td>156</td>
<td>150</td>
<td>6</td>
</tr>
<tr>
<td>Reactive</td>
<td>Non-reactive</td>
<td>11</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Equivocal</td>
<td>Reactive</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Equivocal</td>
<td>Non-reactive</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
<td>Reactive</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
<td>Equivocal</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Non-reactive</td>
<td>Non-reactive</td>
<td>4085</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total Tested</td>
<td>4274</td>
<td>189</td>
<td>183</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Reactive</td>
<td>189</td>
<td>183</td>
<td>172</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* FTA Non-reactive
** Miscellaneous included 2 specimens from patients with arthritis and scleroderma, and one specimen each from patients with Alzheimer, arthrogia: aspergillosis; coeliac disease; coccidioidomycosis; gout; immune complex infection; macroglobulinemia; myeloma (unspecified); myeloma specimen each from patients with Alzheimer; arthralgia; aspergillosis; coeliac disease; colitis C4 - Reactive/FTA Non-reactive

---

**REPRODUCIBILITY**

The reproducibility of CAPTIA™ Syphilis-G was evaluated concurrently at 3 separate US blood/plasma centres. Each centre tested 6 standard serum samples, replicated x 3 in each assay, on each of 5 days. The serum samples comprised: 2 x high titre reactive serum specimens; 2 x low titre (near the cut-off) reactive serum specimens and 2 x non-reactive serum specimens. Results are summarized in the following table:

### CAPTIA SYPHILIS-G SUMMARY OF ASSAY PROCEDURE

**Note:** Read the full product instruction leaflet before starting the assay. This summary is for quick reference only.

1. Dilute 1 part CAPTIA™ Wash Buffer concentrate with 19 parts distilled water. 150 mL is sufficient to wash 1 x 8 well row.
2. Dilute the test sera by adding 50 μL to 1000 μL (1.0 mL) CAPTIA™ Dilution Buffer III in disposable tubes. Do NOT dilute kit controls.
3. Incubation One:
   - Dispense into labeled wells 00 μL of the (i) diluted test sera
   - (ii) Kit Low Titre Reactive Control N DUPLICATE
   - (iii) Kit High Titre Reactive Control
   - (iv) Kit Non Reactive Control
   - 4. Seal the strips with a plate sealer. Incubate at 37(±1)°C for 60(±5) minutes.
   - 5. Aspirate the sera from the wells. Wash the plate five times. Ensure there is no residual fluid in the wells

**Incubation Two:**

- 6. Pipette 100 μL working strength conjugate into each well.
- 7. Seal the strips with a plate sealer. Incubate at 37(±1)°C for 60(±5) minutes.
- 8. Aspirate the conjugate from the wells. Wash the plate five times. Ensure there is no residual wash buffer in the wells.

**Incubation Three:**

- 9. Dispense 100 μL of substrate solution into the wells.
- 10. Incubate at room temperature for 30 (±2) minutes.
- 11. Add 100 μL 1N sulfuric acid to each well. Tap the plate or mix on a plate shaker for 5 to 10 seconds until the blue solution changes to a uniform yellow.

- 12. Within 30 minutes, blank a plate reader on air and read the absorbance of Kit Controls and test sera at 450 nm.

The safety data sheet is available upon request. Caution: Some of the reagents contain ProClin™ at < 0.1% and some contain Sodium Azide at < 0.1%

---

**REFERENCES**

## ORDERING INFORMATION

<table>
<thead>
<tr>
<th>Kit</th>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>800-970</td>
<td>Captia™ Syphilis-G Test Kit</td>
<td>96 Tests</td>
</tr>
<tr>
<td>800-960</td>
<td>Captia™ Syphilis-G Test Kit</td>
<td>960 Tests</td>
</tr>
</tbody>
</table>

### CONTROL

- **+ +** High titre reactive control (HTR)
- **+** Low titre reactive control (LTR)
- **-** Non-reactive control (N)

**Authorized Representative**

Consult accompanying documents

**Manufactured**

**Product Number**

**Lot**

**Use by**

**Caution, consult accompanying documents**

**Store at 2-8°C**

**Store at 2-30°C**

**For In Vitro Diagnostic use**

**Trinity Biotech USA**
Jamestown, NY 14701
Tel. 1 800-325-3424
Fax: 716-488-1990

**Trinity Biotech plc**
Bray Co. Wicklow, Ireland
Tel. 353 1 2769800
Fax 353 1 2769888
www.trinitybiotech.com

800-970-29 Rev F
4/2010