15.4 Transfer 0.05 ml of the mixture to circle 3 and mix as above. Continue the serial dilution through circle 5 and discard 0.05 ml from this last circle after mixing. Circles 1 through 5 represent a dilution series as follows:

<table>
<thead>
<tr>
<th>CIRCLE</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DILUTION</td>
<td>1:16</td>
<td>1:32</td>
<td>1:64</td>
<td>1:128</td>
<td>1:256</td>
</tr>
</tbody>
</table>

15.5 Proceed with the test as described in steps 5 - 9 under the Semi-quantitative Assay Protocol.
15.6 Continue with additional dilutions as required until an endpoint titer is reached.

**LIMITATIONS OF THE PROCEDURE**

16.1 Prozone reactions occur in patients with secondary syphilis. False negative nontreponemal test results, arising from prozone, are also seen in incubating primary and in late syphilis. The prozone reaction is slightly granular or “rough” with specimens exhibiting prozone. When this pattern is exhibited, a dilution of the specimen should be prepared. Titer the diluted specimen until endpoint is reached or until no reactivity is observed. All tests exhibiting a rough appearance should be further evaluated.

16.2 Biological false positive reactions occur occasionally with the CARBON ANTIGEN. Such reactions sometimes occur in samples from individuals with a history of drug abuse, or with diseases such as lupus erythematosus, malaria, sarcoidosis, mononucleosis, leprosy, viral pneumonia, and after smallpox vaccinations.

16.3 Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test.

16.4 Contaminated, icteric, or grossly hemolyzed sera should not be used because of the possibility of nonspecific reactions. A specimen is too hemolyzed for testing when printed matter cannot be read through it.

16.5 Reaction times longer than specified may cause false positive results due to a drying effect.

16.6 Reactive RPR test samples should be substantiated using a confirmatory test as recommended in the Manual of Tests for Syphilis.

16.7 Temperature of the reagents and samples is crucial to test outcome; it should be between 20 and 30°C.

16.8 In accord with all diagnostic methods, a final diagnosis should not be made on the result of a single test, but should be based on a correlation of test results with other clinical findings.

**EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS**

17.1 The Sure-Vue® RPR is evaluated for equivalence, in its pattern of reactivity, against a Reference RPR Card Antigen Suspension.

17.2 A total of 1209 samples were tested by the Sure-Vue® RPR in comparison with the Hyson, Westcott and Dunning (HWD) product. The following results were obtained. There was a 99.2% overall agreement between the two products. Among the 9 samples found to be nonreactive in the HWD product, 8 of the 9 were also nonreactive in the Sure-Vue® RPR test. A 0.05 ml from this last circle after mixing. Circles 1 through 5 represent a dilution series as follows:

<table>
<thead>
<tr>
<th>CIRCLE</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
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<tr>
<td>DILUTION</td>
<td>1:16</td>
<td>1:32</td>
<td>1:64</td>
<td>1:128</td>
<td>1:256</td>
</tr>
</tbody>
</table>

17.3 The nonreactive pattern is slightly granular when compared to the weak reactive. Anti-treponemal antibodies that can be detected by FTA-ABS antigen, and anti-nontreponemal antibodies (reagin) that can be detected by RPR are the target antigens in this test.

17.4 The nonreactive pattern is slightly granular when compared to the weak reactive. Anti-treponemal antibodies that can be detected by FTA-ABS antigen, and anti-nontreponemal antibodies (reagin) that can be detected by RPR are the target antigens in this test.

17.5 The nonreactive pattern is slightly granular when compared to the weak reactive. Anti-treponemal antibodies that can be detected by FTA-ABS antigen, and anti-nontreponemal antibodies (reagin) that can be detected by RPR are the target antigens in this test.

**REFERENCES**

5. Data on file and available on request.
9 SPECIMEN COLLECTION AND STORAGE

- Use heattreated or unheated serum samples, and plasma specimens containing EDTA, CPD, CPDA-1, heparin or sodium citrate as anticoagulants. Plasma specimens should be from tubes or blood units which have been collected with adequate volume to provide the appropriate proportions of specimen to anticoagulant.
- Samples should be free from bacterial contamination, hemolysis, or lipemia.
- Serum samples should be tested within five (5) days of collection. Samples that require longer storage must be removed from the refrigerated and stored at 2-8°C for five (5) days or at -20°C or below until testing 2. Studies have shown that serum samples left on the clot for up to five (5) days at 4°C can be used.
- Plasma samples stored longer than 48 hours should not be used in the assay because of the potential for false reactive results.
- If necessary before testing, centrifuge the specimens at a force sufficient to sediment cellular components.
- Samples to be sent out for testing should be placed on ice packs and packaged like any other biohazardous material that could potentially transmit infection.
- This test should not be used for testing spinal fluids.

10 MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Titer Control (1:16)</th>
<th>100 Tests</th>
<th>500 Tests</th>
<th>5000 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPR CARBON ANTIGEN</td>
<td>n/a</td>
<td>2 ml</td>
<td>9 ml</td>
<td>10 x 9 ml</td>
</tr>
<tr>
<td>REACTIVE CONTROL</td>
<td>1.5 ml</td>
<td>1 ml</td>
<td>1.5 ml</td>
<td>10 x 1.5 ml</td>
</tr>
<tr>
<td>WEAK REACTIVE CONTROL</td>
<td>n/a</td>
<td>1 ml</td>
<td>1.5 ml</td>
<td>10 x 1.5 ml</td>
</tr>
<tr>
<td>NONREACTIVE CONTROL</td>
<td>n/a</td>
<td>1 ml</td>
<td>1.5 ml</td>
<td>10 x 1.5 ml</td>
</tr>
<tr>
<td>3 ml Dropping Bottle</td>
<td>n/a</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>20-ga Dispensing Needle (60 drops/ml)</td>
<td>n/a</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>RPR Test Card (10-well)</td>
<td>n/a</td>
<td>10</td>
<td>50</td>
<td>500*</td>
</tr>
<tr>
<td>0.05 ml Disposable Stirrer Pipets</td>
<td>n/a</td>
<td>100</td>
<td>500</td>
<td>5000</td>
</tr>
</tbody>
</table>

* Also available with 175 (30-well) Test Cards (Cat. No. 23-038020).

11 ADDITIONAL MATERIALS REQUIRED

- Volumetric pipet to deliver 0.05 ml.
- Saline (0.9% NaCl Solution).
- Serum nonreactive to syphilis, in 0.9% saline, for diluting specimens reactive at the 1:16 dilution in the semiquantitative section.
- Mechanical rotator set at 100 ± 5 rpm and circumscribing ¼ inch diameter, with humidity cover.
- Timing Device, minute and second capability.
- Disposable syringes, 1 or 3 ml, accuracy of ± 5%.

12 TEST PROCEDURE

12.1 PREPARATION FOR THE ASSAY

12.1.1 Allow all reagents and samples to warm to room temperature (20-30°C) before use. Remove reagents from foam holders. Do not heat reagents in a water bath.

12.1.2 All reagents are ready for use as supplied. Gently mix the reagents before use; avoid foaming.

12.1.3 Vigorously agitate the CARBON ANTIGEN for 20-30 seconds before each use to ensure homogeneity.

12.2 ASSAY PROTOCOL - QUALITATIVE

12.2.1 Using a stirrer pipet, dispense one free-falling drop (0.05 ml) of serum or plasma sample onto a circle on the test card. Use a fresh stirrer pipet for each sample. When using the stirrer pipet, keep it in a vertical position to ensure accurate delivery. Repeat by adding one free-falling drop of REACTIVE, WEAK REACTIVE OR NONREACTIVE control from the dropper vials supplied. Note the location of each sample by using the numbers located below and to the left of each circle.

12.2.2 Using the flat end of the stirrer pipet, spread the sample over the entire area of the test circle. Do not scratch the surface of the test area.

12.2.3 Attach the needle to the dropping bottle. Mix the CARBON ANTIGEN suspension well. Squeeze the dropping bottle and draw a sufficient volume of the antigen suspension into the bottle. Dispense several drops into the test area. DO NOT RESTIR the sample and the antigen. Aspirate any antigen from the bottle cap and return to dispensing bottle.

12.2.4 Prior to dispensing carbon antigen, agitate the dropping bottle for a few seconds to ensure reagent homogeneity. Dispense one free-falling drop of the antigen suspension onto each sample while holding the bottle in a vertical position. DO NOT RESTIR the sample and the antigen. Aspirate any antigen from the bottle cap and return to dispensing bottle.

12.2.5 Place the card on an automatic rotator and cover to maintain humidity. Rotate at 100 ± 5 rpm for 8 minutes (7 minutes 50 seconds to 8 minutes 30 seconds). Following rotation, a brief hand rotation and tilting of the card (3-4 times) should be performed to aid in differentiating nonreactive from minimally reactive results.

12.2.6 Immediately read results macroscopically in the “wet” state under a high intensity light source.

12.2.7 Remove and wash the needle at the end of each test run.

12.3 ASSAY PROTOCOL - SEMIQUANTITATIVE

12.3.1 Using a stirrer pipet (or other accurate volumetric pipet capable of delivering 0.05 ml), dispense one free-falling drop of saline onto circles to be numbered 2 to 5. DO NOT SPREAD.

12.3.2 Using the stirrer pipet (or other accurate volumetric pipet capable of delivering 0.05 ml), dispense one free-falling drop of serum or plasma sample onto circle 1 on the test card. DO NOT SPREAD.

12.3.3 Using an accurate volumetric pipet (not a stirrer pipet), dispense 0.05 ml of the test sample onto circle 2. Insert the tip of the pipet into the resulting mixture and mix by carefully drawing the mixture up and down in the pipet 5 or 6 times. Avoid any bubble formation.

12.3.4 Transfer 0.05 ml of the mixture in circle 2 to circle 3 and mix. Repeat this serial dilution procedure to circle 4 and then circle to 5; discard 0.05 ml from this last circle. Circles 1 through 5 now represent a dilution series as follows:

<table>
<thead>
<tr>
<th>DILUTION</th>
<th>1:1</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIRCLE</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

12.3.5 Using the flat end of the stirrer pipet, spread the diluted samples over the entire areas of the test circles, starting at circle 3 (highest dilution). Repeat this spreading procedure in circles 4 through 1.

12.3.6 Attach the needle to the dropping bottle. Mix the CARBON ANTIGEN suspension well. Squeeze the dropping bottle and draw a sufficient volume of the antigen suspension into the bottle. Dispense several drops into the test area. DO NOT RESTIR the sample and the antigen. Aspirate any antigen from the bottle cap. Do not scratch the surface of the test area.

12.3.7 Prior to dispensing carbon antigen, agitate the dropping bottle for a few seconds to ensure reagent homogeneity.

12.3.8 Dispense one free-falling drop of the antigen suspension onto each sample while holding the bottle in a vertical position. DO NOT RESTIR the sample and the antigen. Aspirate any antigen from the bottle cap. Do not scratch the surface of the test area.

12.3.9 Immediately read results macroscopically in the “wet” state under a high intensity light source.

12.3.10 Remove and wash the needle at the end of each test run.

13 QUALITY CONTROL

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control Procedures. Controls with graded reactivity should be included. If control samples do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the control samples, discontinue use of the kit.

14 INTERPRETATION OF RESULTS

14.1 INTERPRETATION OF RESULTS - QUALITATIVE

14.1.1 A reactive result is indicated by the presence of aggregates in the center or periphery of the test circle, ranging from slight to marked and intense.

14.1.2 A nonreactive result will give a smooth gray appearance within the test circle or a button of non-aggregated carbon particles in the center of the circle, showing none of the clumping characteristics of a reactive result.

14.2 Results for the Sure-Vue® RPR test should be reported only as Reactive or Nonreactive, regardless of the degree of reactivity. Minimal to moderate reactivity should always be reported as Reactive.

14.3 Slightly granular or “rough” reactions should be repeated using an alternative procedure. For donor screening, those results should be reported as “Indeterminate” pending further evaluation. See the “Limitations of the Procedure” section.

14.4 If necessary, confirm reactive results by retesting the sample using the semiquantitative procedure.

15 SAMPLES WITH TITERS GREATER THAN 1:16

15.1 Prepare a 1:16 dilution of nonreactive serum in saline. This is to be used for making 1:32 and higher dilutions of samples to be titered. Dispense 0.05 ml of this solution onto circles labeled 2 through 5. DO NOT SPREAD.

15.2 Prepare a 1:16 dilution of sample test by adding 0.1 ml of serum to 1.5 ml of saline. Mix thoroughly. Dispense 0.05 ml of this diluted sample onto circles 1 and 2. Do not spread.

15.3 Mix the solution on circle 2 by drawing the solution up and down 5 or 6 times into the tip of a volumetric pipet. Avoid any bubble formation.
9 SPECIMEN COLLECTION AND STORAGE

9.1 Use heattreated or unheated serum samples, and plasma specimens containing EDTA, CPD, CPDA-1, heparin or sodium citrate as anticoagulants. Plasma specimens should be from tubes or blood units which have been collected with adequate volume to provide the appropriate proportions of specimen to anticoagulant.

9.2 Samples should be free from bacterial contamination, hemolysis, or lypemia.

9.3 Serum samples should be tested within five (5) days of collection. Samples that require longer storage must be removed from the red cells and may be stored at 2-8°C for five (5) days or at -20°C or below until testing. Studies have shown that serum samples left on the clot for up to five (5) days at 40°C can be used.

9.4 Plasma samples stored longer than 48 hours should not be used in the assay because of the potential for false reactive results.

9.5 If necessary before testing, centrifuge the specimens at a force sufficient to sediment cellular components.

9.6 Samples to be sent out for testing should be placed on ice packs and packaged like any other biohazardous material that could potentially transmit infection.

9.7 This test should not be used for testing spinal fluids.

10 MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Titer Control (1:16)</th>
<th>100 Tests</th>
<th>500 Tests</th>
<th>5000 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPR CARBON ANTIGEN</td>
<td>n/a</td>
<td>2 ml</td>
<td>9 ml</td>
</tr>
<tr>
<td>REACTIVE CONTROL</td>
<td>1.5 ml</td>
<td>1 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>WEAK REACTIVE CONTROL</td>
<td>n/a</td>
<td>1 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>NONREACTIVE CONTROL</td>
<td>n/a</td>
<td>1 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>3 ml Dropping Bottle</td>
<td>n/a</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>20-ga Dispensing Needle (60 drops/ml)</td>
<td>n/a</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>RPR Test Card (10-well)</td>
<td>n/a</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>0.05 ml Disposable Stirrer Pipets</td>
<td>n/a</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>* Also available with 175 (30-well) Test Cards (Cat. No. 23-038020).</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cat. No. 23-038011, 9 ml RPR Carbon Antigen.
Cat. No. 23-038012, 1.5 ml Reactive control, 1.5 ml Weak Reactive control and 1.5 ml Nonreactive control.
Cat. No. 23-038013, 50 RPR test cards (10-well).
Cat. No. 23-038014, 25 RPR Test Cards (30-well).
Cat. No. 23-038015, 500 Disposable Stirrer Pipets (0.05 ml).

11 ADDITIONAL MATERIALS REQUIRED

11.1 Volumetric pipet to deliver 0.05 ml.

11.2 Saline (0.9% NaCl Solution).

11.3 Serum nonreactive to syphilis, in 0.9% saline, for diluting specimens reactive at the 1:16 dilution in the semiquantitative section.

11.4 Mechanical rotator set at 100 ± 5 rpm and circumscribing ¾ inch diameter, with humidity cover.

11.5 Timing Device, minute and second capability.

11.6 Disposable syringes, 1 or 3 ml, accuracy of ± 5%.

12 TEST PROCEDURE

12.1 PREPARATION FOR THE ASSAY

12.1.1 Allow all reagents and samples to warm to room temperature (20-30°C) before use. Remove reagents from foil holders. Do not heat reagents in a water bath.

12.1.2 All reagents are ready for use as supplied. Gently mix the reagents before use; avoid foaming.

12.1.3 Vigorously agitate the CARBON ANTIGEN for 30 seconds before each use to ensure homogeneity.

12.2 ASSAY PROTOCOL - QUALITATIVE

12.2.1 Using a stirrer pipet, dispense one free-falling drop (0.05 ml) of serum or plasma sample onto a circle on the test card. Use a fresh stirrer pipet for each sample. When using the stirrer pipet, keep it in a vertical position to ensure accurate delivery. Repeat by adding one free-falling drop of reactive, weak reactive or nonreactive control from the dropper vials supplied. Note the location of each sample by using the numbers located below and to the left of each circle.

12.2.2 Using the flat end of the stirrer pipet, spread the sample over the entire area of the test circle. Do not scratch the surface of the test area.

12.2.3 Attach the needle to the dropping bottle. Mix the CARBON ANTIGEN suspension well. Squeeze the dropping bottle and draw a sufficient volume of the antigen suspension into the bottle. Disperse several drops into the antigen suspension onto each sample while holding the bottle in a vertical position. DO NOT RESTIR the sample and the antigen. Aspirate any antigen from the bottle cap and return to dispersing bottle.

12.2.4 Prior to dispensing carbon antigen, agitate the dropping bottle for a few seconds to ensure reagent homogeneity. Dispense one free-falling drop of the antigen suspension onto each sample while holding the bottle in a vertical position. DO NOT RESTIR the sample and the antigen. Aspirate any antigen from the bottle cap and return to dispersing bottle.

12.2.5 Place the card on an automatic rotator and cover to maintain humidity. Rotate at 100 ± 5 rpm for 8 minutes (7 minutes 50 seconds to 8 minutes 30 seconds). Following rotation, a brief hand rotation of the card (3-4 times) should be performed to aid in differentiating nonreactive from minimally reactive results.

12.2.6 Immediately read results macroscopically in the "wet" state under a high intensity light source.

12.2.7 Remove and wash the needle at the end of each test run.

12.3 ASSAY PROTOCOL - SEMIQUANTITATIVE

12.3.1 Using a stirrer pipet (or other accurate volumetric pipet capable of delivering 0.05 ml), dispense one free-falling drop of saline onto circles to be numbered 2 to 5. DO NOT SPREAD.

12.3.2 Using the stirrer pipet (or other accurate volumetric pipet capable of delivering 0.05 ml), dispense one free-falling drop of serum or plasma sample onto circle 1 on the test card. DO NOT SPREAD.

12.3.3 Using an accurate volumetric pipet (not a stirrer pipet), dispense 0.05 ml of the test sample onto circle 2. Insert the tip of the pipet into the resulting mixture and mix by carefully drawing the mixture up and down in the pipet 5 or 6 times. Avoid any bubble formation.

12.3.4 Transfer 0.05 ml of the mixture in circle 2 to circle 3 and mix. Repeat this serial dilution procedure to circle 4 and then to circle 5; discard 0.05 ml from this last circle. Circles 1 through 5 now represent a dilution series as follows:

<table>
<thead>
<tr>
<th>CIRCLE</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DILUTION</td>
<td>1:1</td>
<td>1:2</td>
<td>1:4</td>
<td>1:8</td>
<td>1:16</td>
</tr>
</tbody>
</table>

12.3.5 Using the flat end of the stirrer pipet, spread the diluted samples over the entire areas of the test circles, starting at circle 5 (highest dilution). Repeat this spreading procedure in circles 4 through 1.

12.3.6 Attach the needle to the dropping bottle. Mix the CARBON ANTIGEN suspension well. Squeeze the dropping bottle and draw a sufficient volume of the antigen suspension into the bottle. Disperse several drops into the antigen suspension onto each sample while holding the bottle in a vertical position. DO NOT RESTIR the sample and the antigen. Aspirate any antigen from the bottle cap.

12.3.7 Place the card on the automatic rotator and cover to maintain humidity. Rotate at 100 ± 5 rpm for 8 minutes (7 minutes 50 seconds to 8 minutes 30 seconds). Following rotation, a brief hand rotation and tilting of the card (3-4 times) should be performed to aid in differentiating nonreactive from minimally reactive results.

12.3.8 Prior to dispensing carbon antigen, agitate the dropping bottle for a few seconds to ensure reagent homogeneity. Dispense one free-falling drop of the antigen suspension onto each sample while holding the bottle in a vertical position. DO NOT RESTIR the sample and the antigen. Aspirate any antigen from the bottle cap.

12.3.9 Immediately read results macroscopically in the "wet" state under a high intensity light source.

12.3.10 Remove and wash the needle at the end of each test run.

13 QUALITY CONTROL

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control Procedures. Controls with graded reactivity should be included. If controls do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the control samples, discontinue use of the kit.

14 INTERPRETATION OF RESULTS

14.1 INTERPRETATION OF RESULTS - QUALITATIVE

14.1.1 A reactive result is indicated by the presence of aggregates in the center or periphery of the test circle, ranging from slight to marked and intense.

14.1.2 A nonreactive result will give a smooth gray appearance within the test circle or a button of non-aggregated carbon particles in the center of the circle, showing none of the clumping characteristics of a reactive result.

14.1.3 Results for the Sure-Vue® RPR test should be reported only as Reactive or Nonreactive, regardless of the degree of reactivity. Minimal to moderate reactivity should always be reported as Reactive.

14.1.4 Slightly granular or "rough" reactions should be repeated using an alternative procedure. For donor screening, these should be reported as "indeterminate" pending further evaluation. See the "Limitations of the Procedure" section.

14.1.5 If necessary, confirm reactive results by retesting the sample using the semiquantitative procedure.

14.2 INTERPRETATION OF RESULTS - SEMIQUANTITATIVE

The highest dilution in which visible aggregation occurs is the endpoint titer.

15 SAMPLES WITH TITERS GREATER THAN 1:16

15.1 Prepare a 1:50 dilution of nonreactive serum in saline. This is to be used for making 1:32 and higher dilutions of samples to be titrated. Dispense 0.05 ml of this solution onto circles labeled 2 through 5. DO NOT SPREAD.

15.2 Prepare a 1:16 dilution of test sample by adding 0.1 ml of serum to 1.5 ml of saline. Mix thoroughly. Dispense 0.05 ml of this diluted sample onto circles labeled 1 and 2. DO NOT SPREAD.

15.3 Mix the solution on circle 2 by drawing the solution up and down 5 or 6 times into the tip of a volumetric pipet. Avoid any bubble formation.
15.4 Transfer 0.05 ml of the mixture to circle 3 and mix as above. Continue the serial dilution through circle 5 and discard 0.05 ml from this last circle after mixing. Circles 1 through 5 represent a dilution series as follows:

<table>
<thead>
<tr>
<th>CIRCLE</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DILUTION</td>
<td>1:16</td>
<td>1:32</td>
<td>1:64</td>
<td>1:128</td>
<td>1:256</td>
</tr>
</tbody>
</table>

15.5 Proceed with the test as described in steps 5 - 9 under the Semi-Quantitative Assay Protocol.

15.6 Continue with additional dilutions as required until an endpoint titer is reached.

16 LIMITATIONS OF THE PROCEDURE

16.1 Prozone reactions occur in patients with secondary syphilis. False negative nontreponemal test results, arising from prozone, are also seen in incubating primary and in late syphilis. The prozone reaction is slightly granular or "rough" with specimens exhibiting prozone. When this pattern is exhibited, a dilution of the specimen should be prepared. Titers the diluted specimen until endpoint is reached or until no reactivity is observed. All tests exhibiting a rough appearance should be further evaluated.

16.2 Biological false positive reactions occur occasionally with the CARBON ANTIGEN. Such reactions sometimes occur in samples from individuals with a history of drug abuse, or with diseases such as lupus erythematosus, malaria, vaccinia, mononucleosis, leprosy, viral pneumonia, and after smallpox vaccinations.

16.3 Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test.

16.4 Contaminated, infecric, or grossly hemolyzed sera should not be used because of the possibility of nonspecific reactions. A specimen is too hemolyzed for testing when printed matter cannot be read through it.

16.5 Reaction times longer than specified may cause false positive results due to a drying effect.

16.6 Reactive RPR test samples should be substantiated using a confirmatory test as recommended in the Manual of Tests for Syphilis.

16.7 Temperature of the reagents and samples is crucial to test outcome; it should be between 20 and 30°C.

16.8 In accord with all diagnostic methods, a final diagnosis should not be made on the result of a single test, but should be based on a correlation of test results with other clinical findings.

17 EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS

17.1 The Sure-Vue® RPR is evaluated for equivalence, in its pattern of reactivity, against a Reference RPR Card Antigen Suspension.

17.2 A total of 1209 samples were tested by the Sure-Vue® RPR in comparison with the Hynson, Westcott and Dunning (HWD) product. The following results were obtained. There was a 99.2% overall agreement between the two products. Among the 9 samples found to be nonreactive in the Sure-Vue® RPR test, 7 were also confirmed negative by the FTA-ABS test.

<table>
<thead>
<tr>
<th>Sure-Vue® RPR</th>
<th>HWD RPR TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>462</td>
</tr>
<tr>
<td>Nonreactive</td>
<td>2</td>
</tr>
<tr>
<td>Reactive</td>
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</tr>
<tr>
<td>Nonreactive</td>
<td>15</td>
</tr>
<tr>
<td>Nonreactive</td>
<td>177</td>
</tr>
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</table>

18 REFERENCES

5. Data on file and available on request.