Title: **ASI RPR CARD TEST FOR SYPHILIS**		Doc#: 6004-900 CLSI
Effective Date: 05/2023	Supersedes Revision/Date: 03/2017	Revision: 05/2023

For in vitro diagnostic use

Catalog Number	***Kit Size***
900025	**25 Tests**
900100	***100 Tests***
900500	***500 Tests***
9005000	***5000 Tests***
90010000	**10000 Tests**

CPT Code: 86592

1 INTENDED USE: The **ASI RPR** (rapid plasma reagin) **Card Test** for syphilis is a qualitative and semiquantitative nontreponemal flocculation test for the detection of reagin antibodies in human serum and plasma as a screening test for serological evidence of syphilis. This test is also intended for use in screening blood donors and cadaveric (non-heart beating) donor specimens for tissue donation when the test is read and interpreted with the ASiManager-AT. The ASI RPR Card Test for Syphilis is for professional use only.

2 SUMMARY AND EXPLANATION: *Treponema pallidum*, the etiological agent of syphilis, induces the production of at least two types of antibodies in human infection: anti-treponemal antibodies that can be detected by FTA-ABS antigen[1], and anti-nontreponemal antibodies (reagin) that can be detected by RPR antigen[2].

3 PRINCIPLE OF THE PROCEDURE: The **ASI RPR Card Test** is an 8-minute macroscopic nontreponemal flocculation test to be used for the detection of reagin. The microparticulate carbon RPR antigen enhances the visual discrimination between reactive and nonreactive results. The reagin-type antibody binds with the antigen that is composed of a complex of cardiolipin, lecithin and cholesterol particles with activated charcoal. The result of this antigen-antibody reaction is macroscopic flocculation.

4 REAGENTS

4.1 CARBON ANTIGEN - 0.003% cardiolipin, 0.020–0.022% lecithin, 0.09% cholesterol, charcoal (activated) as visual enhancer, phosphate buffer, 0.1% sodium azide as preservative and stabilizer.

4.2 CONTROLS (REACTIVE, WEAK REACTIVE, NONREACTIVE) - Human serum or defibrinated plasma (liquid), with 0.1% sodium azide as preservative.

5 WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

5.1 **ASI RPR REAGENTS** contain sodium azide. Azides in contact with lead and copper plumbing may react to form highly explosive metal azides. When disposing of reagents containing azide, flush down the drain with large quantities of water to prevent azide buildup.

5.2 **ASI RPR CONTROLS** contain human serum or plasma which has been tested at the donor level for HBsAg and for HIV-1, HIV-2 and HCV antibodies and found to be nonreactive. As no known test offers complete assurance that infectious agents are absent, the CONTROLS should be considered potentially infectious and universal precautions should be used. The CDC/NIH Health Manual “Biosafety in Microbiological and Biomedical Laboratories” describes how these materials should be handled in accordance with Good Laboratory Practice.

5.3 Do not pipet by mouth.

5.4 Do not smoke, eat, drink or apply cosmetics in areas where plasma/serum samples are handled.

5.5 Any cuts, abrasions or other skin lesions should be suitably protected.

6 HANDLING AND PROCEDURAL NOTES

6.1 In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.

6.2 ASI RPR test cards are plastic coated and specifically designed to be used with the RPR antigen. In handling, take care not to fingermark the card areas, as this may result in an oily deposit and improper test result. When spreading specimen within the confines of the circle area, avoid scratching the card with the stirrer pipets. If the specimen does not spread in the test area or spreads outside the test area, use another test circle.

6.3 The needle assembly must be thoroughly washed in distilled or deionized water and air dried after each shift. **Do not wipe the needle dry**. Place the needle back into the plastic sleeve. Do not remove bottle tip when

washing the needle assembly. Let the assembly air dry. Before next use, make sure that no large water droplets remain in the dropping bottle by shaking the bottle and squeezing it.

6.4 The needle should deliver 60 ± 2 drops of antigen suspension per milliliter when held in a vertical position. To perform accuracy check on the needle, attach the needle to a 1 or 3 ml syringe. Fill the syringe with the antigen suspension and, holding the syringe in a vertical position, count the number of drops delivered in 0.5 ml. The needle is considered satisfactory if 30 ± 1 drops are obtained in 0.5 ml.

6.5 Do not use past the expiration date indicated on the kit.

6.6 Do not interchange components from this kit with those of a different manufacturer. Discard the dispensing needle and dropping bottle when the carbon antigen is exhausted.

7 STORAGE INSTRUCTIONS

7.1 Store all reagents at 2–8°C in an upright position when not in use. Do not freeze reagents. Pipets and cards do not require refrigeration. Carbon Antigen may be stored for up to one month in the dropping bottle at 2–8°C; in this case, the needle must be cleaned at the end of each shift, using a syringe or pipet.

8 INDICATIONS OF DETERIORATION

8.1 Turbidity or precipitation in controls is indicative of deterioration and the component should not be used.

8.2 Bacterial contamination of reagents or specimens may cause false positive results.

9 SPECIMEN COLLECTION AND STORAGE

9.1 Specimens for visual reading
Use heated or unheated serum samples, and plasma specimens containing EDTA[3, 4,] CPD, CPDA-1, heparin or sodium citrate[5] 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, gross hemolysis, or lipemia. A specimen is too hemolyzed for testing when printed matter cannot be read through it[2].

9.3 Serum samples should be tested within five (5) days of collection. Optimum storage temperature for samples is 2-8°C. Samples that require longer than five (5) days storage must be removed from the red cells and stored at -20°C or below until testing[2].

9.4 If needed, studies have shown that serum samples left on the clot for up to five (5) days at 45°C can be used. If the sample is hemolyzed, see item 3 above[5].

9.5 Plasma samples stored longer than five (5) days at 2-8°C should not be used in the assay because of the potential for false reactive results.

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

9.7 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.

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

10 PERFORMANCE OF THE TEST

Materials Provided:

	25 Tests	100 Tests	500 Tests*	5000 Tests*	10000 Tests*
RPR CARBON ANTIGEN	0.5 ml	2.0 ml	9.0 ml	10 x 9.0 ml	20 x 9.0 ml
REACTIVE CONTROL	0.5 ml	1.0 ml	2.0 ml	5 x 2.0 ml	5.0 ml
WEAK REACTIVE CONTROL	0.5 ml	1.0 ml	2.0 ml	5 x 2.0 ml	5.0 ml
NONREACTIVE CONTROL	0.5 ml	1.0 ml	2.0 ml	5 x 2.0 ml	5.0 ml
3 ml Dropping Bottle	1	1	1	10	10
20-ga Dispensing Needle (60 drops/ml)	1	1	1	10	10
RPR Test Card (10-well)	3	10	50	500	n/a
RPR Test Card (15-well)	n/a	n/a	35	350	675
RPR Test Card (30-well)	n/a	n/a	n/a	175	350
0.05 ml Disposable Stirrer Pipets	25	100	500	5000	10000

*Also available without controls

Additional Materials Required

10.1 Volumetric pipet to deliver 0.05 ml.

10.2 Saline (0.9% NaCl Solution).

10.3 Serum nonreactive to syphilis, in 0.9% saline, for diluting specimens reactive at the 1:16 dilution in the semiquantitative procedure.
10.4 Mechanical rotator set at 100 ± 5 rpm and circumscribing ¾ inch diameter, with humidity cover.
10.5 Timing device, minute and second capability.
10.6 Disposable syringe, 1 or 3 ml, accuracy of ± 5%.

11 PREPARATION FOR THE ASSAY

11.1 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.

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

11.3 Vigorously agitate the CARBON ANTIGEN for 20–30 seconds before each use in order to ensure homogeneity.

12 ASSAY PROTOCOL – QUALITATIVE

12.1 Using a stirrer pipet, dispense one free-falling drop (0.05 ml) of each serum or plasma sample onto a separate 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 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.

12.3 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 dropping bottle cap to make sure the needle passage is clear.

12.4 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.

12.5 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 (at least 3–4 times) should be performed to aid in differentiating nonreactive from minimally reactive results.

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

12.7 Remove and wash the needle at the end of each shift.

13 ASSAY PROTOCOL – SEMIQUANTITATIVE

13.1 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.

13.2 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.

13.3 Using an accurate volumetric 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 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.

13.4 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:

CIRCLE	1	2	3	4	5
DILUTION	1:1	1:2	1:4	1:8	1:16

13.5 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 the spreading procedure in circles 4 through 1.

13.6 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 dropping bottle cap to make sure the needle passage is clear.

13.7 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.

13.8 Place the card onto 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.

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

13.10 Remove and wash the needle at the end of each shift.

14 QUALITY CONTROL

14.1 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 and contact ASI Technical Support at 800-654-0146.

15 VISUAL INTERPRETATION OF RESULTS – QUALITATIVE

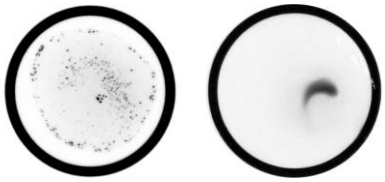
15.1 A reactive result is indicated by the presence of aggregates in the center or periphery of the test circle, ranging from slight to marked and intense. A nonreactive result will give a smooth gray appearance within the test circle or a button of nonaggregated carbon particles in the center of the circle, showing none of the clumping characteristics of a reactive result.

15.2 Results for the ASI RPR Card 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.

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

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

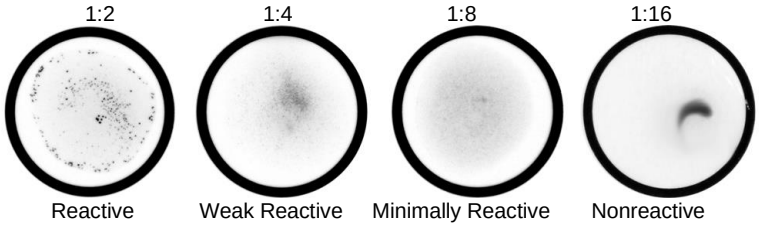
15.5 It is not necessary to perform the quantitative procedure on reactive donor samples.



Reactive Nonreactive

16 VISUAL INTERPRETATION OF RESULTS - SEMIQUANTITATIVE

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



17 SAMPLES WITH TITERS GREATER THAN 1:16

17.1 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 titered. Dispense 0.05 ml of this solution onto circles labeled 2 through 5. DO NOT SPREAD.

17.2 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 1 and 2. DO NOT SPREAD.

17.3 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.

17.4 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:

CIRCLE	1	2	3	4	5
DILUTION	1:16	1:32	1:64	1:128	1:256

17.5 Proceed with the test as described in steps 13.5 through 13.9 under the "Semiquantitative Assay Protocol".

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

18 LIMITATIONS OF THE PROCEDURE

19.1 Prozone reactions occur in patients with secondary syphilis[6]. False negative nontreponemal test results, arising from prozone, are also seen in incubating primary and in late syphilis[2]. The nonreactive pattern 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.

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

19.3 Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test[2].

19.4 Contaminated, lipemic, 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[2].

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

19.6 Reactive RPR test samples should be substantiated using a confirmatory test as recommended in the Manual of Tests for Syphilis[2,7].

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

19.8 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.

20 PERFORMANCE CHARACTERISTICS

20.1 The ASI RPR Card Test is evaluated for equivalence, in its pattern of reactivity, against a reference RPR card antigen suspension. A total of 1209 samples were tested by the ASI RPR Card Test in comparison with the Hynson, Westcott and Dunning (HWD) product[8]. 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 ASI test, 7 were also confirmed negative by the FTA-ABS test.

		ASI RPR TEST	
		Reactive	Nonreactive
HWD RPR TEST	Reactive	462	9
	Nonreactive	1	737

21 REFERENCES

1. Hunter EF, Deacon WE, Myer PE. 1964. *Public Health Reports*, **79**:410-412.
2. Larsen SA, Hunter EF, Kraus SJ (ed.). 1990. *Manual of Tests for Syphilis*, Public Health Service, Washington, D.C.
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4 Dyckman JD and Wende RD. 1980. *J Clin Microbiol*, **11**:16-18.
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8. Fischer GS, Colavita MT, Sweimler WI, Kleger B. 1989. *J Clin Microbiol*, 27:188-189.

TECHNICAL INFORMATION: (801) 489-8911 or (800) 654-0146