ARLINGTON SCIENTIFIC

ASI RPR Test for Syphilis For Use on the ASI Evolution®

This test is intended for use in screening blood donors.

For in vitro diagnostic use	Rx Only
Catalog Number	**Kit Size**
900480ABB	480 Tests
9004800ABB	4800 Tests
CPT Code 86592 (Screening)	

INTENDED USE

The ASI Automated RPR (rapid plasma reagin) Test for Syphilis, for use on the ASI Evolution, is a qualitative nontreponemal flocculation test for the detection of reagin antibodies in human serum and plasma as a screening test for serological evidence of syphilis.

The ASI Evolution® is intended to be used as a fully automated analyzer to objectively interpret the results of the ASI Automated RPR Test for Syphilis. The ASI Evolution is designed to provide standardized test interpretation and to provide for storage, retrieval, and transmittal of the test results.

The ASI Automated RPR Test for Syphilis for use on the ASI Evolution is for professional use only. The test is intended to be used for blood donor screening. This test is not intended for diagnostic use.

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].

PRINCIPLE OF THE PROCEDURE

The **ASI Automated RPR Test for Syphilis for use on the ASI Evolution** is an automated macroscopic nontreponemal flocculation test to be used for the detection of reagin. This test kit is intended to be used with the ASI Evolution Automated Syphilis Analyzer. The ASI Evolution instrument automates the dispensing of serum or plasma samples and the dispensing of carbon antigen reagent. 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. The ASI Evolution uses an internal camera and image processing algorithm to read the RPR agglutination reaction and report a reactive or nonreactive result.

REAGENTS

- **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 stabilizers.
- **CONTROLS** (REACTIVE, WEAK REACTIVE, NONREACTIVE) - Human serum or defibrinated plasma (liquid), with 0.1% sodium azide as preservative.
- **REAGENTS** have two-year expiration dating from date of manufacture. The specific expiration date is located on the label on the vial.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

1. **ASI AUTOMATED 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.
2. **ASI AUTOMATED 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.
3. Do not pipet by mouth.
4. Do not smoke, eat, drink or apply cosmetics in areas where plasma/serum samples are handled.
5. Any cuts, abrasions or other skin lesions should be suitably protected.

HANDLING AND PROCEDURAL NOTES

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.
2. Do not use past the expiration date indicated on the kit.
3. Do not interchange components from this kit with those of a different manufacturer.

STORAGE INSTRUCTIONS
Store all reagents at 2–8°C in an upright position when not in use. Do not freeze reagents.

INDICATIONS OF DETERIORATION
1. Turbidity or precipitation in controls is indicative of deterioration and the component should not be used.
2. Bacterial contamination of reagents or specimens may cause false positive results.

SPECIMEN COLLECTION AND STORAGE
1. Use either serum or Sodium Citrate plasma specimens for testing with the **ASI Automated RPR Test for Syphilis on the ASI Evolution** instrument; the use of other anticoagulants has not been evaluated.
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].
3. Serum samples should be tested within five (5) days of collection. Samples should be stored at 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].
4. 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.
5. If necessary before testing, centrifuge the specimens at a force sufficient to sediment cellular components.
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.
7. This test should not be used for testing spinal fluids.

PERFORMANCE OF THE TEST

Materials Provided:

	480 Tests	4800 Tests
RPR CARBON ANTIGEN	23 ml	23 ml x 10
REACTIVE CONTROL	2.0 ml	2.0 ml x 10
WEAK REACTIVE CONTROL	2.0 ml	2.0 ml x 10
NONREACTIVE CONTROL	2.0 ml	2.0 ml x 10
MICROWELL PLATES (48 WELL)	10	100

TEST PROCEDURE - Qualitative
1. Create or select a work list.
2. Load samples as work list is created.
3. Load carbon antigen reagent. Vigorously agitate the carbon antigen for 20-30 seconds before placing the vial into the reagent rack. Ensure that stir bar is in vial.
4. Select test to perform. R/NR for qualitative testing.
5. Name work list.
6. Close Cover
7. Press start.
8. Dispose of used microtiter plates in accordance with federal (40 CFR 261.3), state, local or Good Laboratory Practice requirements.

See Operator's Manual for complete instructions.

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

LIMITATIONS OF THE PROCEDURE
1. Prozone reactions can occur in patients with secondary syphilis[5]. False negative nontreponemal test results, arising from prozone, can also be 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.
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.
3. Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test[2].
4. The cover of the ASI Evolution should be closed while tests are being performed to avoid glare from outside lighting sources.
5. Temperature of the reagents and samples is crucial to test outcome; it should be between 20-30°C.
6. The ASI Evolution must have the exposure calibrated before use. Refer to section 4.4.2 of the Operator's Manual for complete instructions. The calibration should be done every 3 months.

PERFORMANCE CHARACTERISTICS
The ASI Evolution was evaluated for equivalence in its pattern of reactivity against the ASiManager-AT. A total of 2,861 individual samples in 3,757 tests were conducted by the ASI Evolution compared with the ASiManager-AT, including a panel of 448 samples of known reactivity was tested at three sites: they yielded the same results and were counted only once in the statistical analysis. The distribution of the samples to the sites is represented in Table 1. The results were broken down for serum and plasma samples in Tables 2 and 3 respectively.

Table 1

Site	Prospective random samples		Retrospcetive samples[3]				A panel of known reactivity[3]		Total
			Plasma		Serum		Serum		
	Plasma[1]	Serum[2]	Known infected	Known uninfected	Known infected	Known uninfected	Known infected	Known uninfected	
A	500	0	0	0	0	0	400	48	948
B	100	400	0	0	0	0	400	48	948
C	0	0	410	993	7	3	400	48	1,861
Total	**600**	**400**	**410**	**993**	**7**	**3**	**1200**	**144**	**3,757**

1 There were 3 concordant reactive results.
2 There were 9 concordant reactive results and 4 discordant results (ASI Evolution nonreactive and ASiManager-AT reactive).
3 All tests gave the expected results.

Combined prospective and retrospective (including one panel) results of testing serum samples on the ASI Evolution and the ASiManager-AT

Table 2

		ASI Evolution		
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	416	4	420
	Nonreactive	0	438	438
	Total	416	442	858

The 4 discordant results (ASI Evolution nonreactive and ASiManager-AT reactive) were found to be nonreactive when tested with a treponemal test and a nontreponemal test. Below are the calculations for positive percentage agreement (PPA) and negative percentage agreement (NPA) including 95% confidence interval (CI) for serum samples.

PPA = 416/420 = 99.05% 95% CI: (97.58%, 99.74%)
NPA = 438/438 = 100% 95% CI: (99.16%, 100%)

ASI Evolution specificity for serum samples was calculated using prospective random samples excluding 9 confirmed positive samples (Table 1, footnote 2): 400 - 9 = 391. Sensitivity was calculated using known infected serum samples (Table 1): 400 + 7 = 407.

Specificity = 391/391 = 100% 95% CI: (99.03%, 100%)
Sensitivity = 407/407 = 100% 95% CI: (99.10%, 100%)

Combined prospective and retrospective results of testing plasma samples on the ASI Evolution and the ASiManager-AT

Table 3

		ASI Evolution		
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	413	0	413
	Nonreactive	0	1590	1590
	Total	413	1590	2003

Below are the calculations for PPA and NPA including 95% CI for plasma samples.

PPA = 413/413 = 100% 95% CI: (99.11%, 100%)
NPA = 1590/1590 = 100% 95% CI: (99.77%, 100%)

ASI Evolution specificity for plasma samples was calculated using prospective random samples excluding 3 confirmed positive samples (Table 1, footnote 1): 600 - 3 = 597. Sensitivity was calculated using 410 known infected plasma samples (Table 1).

Specificity = 597/597 = 100% 95% CI: (99.38%, 100%)
Sensitivity = 410/410 = 100% 95% CI: (99.10%, 100%)

A total of 10 samples were evaluated to determine reproducibility of reactivity between three instruments. Of the 10 samples, 7 were reactive and 3 were nonreactive. The reactive samples had titers ranging from 1:1 to 1:256. Each of the 10 samples was analyzed once in each well of four different plates on each of the three instruments to evaluate the reactivity. The data are shown in Table 4.

Table 4

	Sample	Expected	Results						% Agreement
	Sample ID	Titer	Evolution 1		Evolution 2		Evolution 3		
1	R7C21R	1:8	R	192/192	R	192/192	R	192/192	100%
2	N7D04	NR	NR	192/192	NR	192/192	NR	192/192	100%
3	11114B	1:1	R	192/192	R	192/192	R	192/192	100%
4	11114C	1:1	R	192/192	R	192/192	R	192/192	100%
5	11114F	1:1	R	192/192	R	192/192	R	192/192	100%
6	02287	NR	NR	192/192	NR	192/192	NR	192/192	100%
7	08296	1:256	R	192/192	R	192/192	R	192/192	100%
8	11114D	1:1	R	192/192	R	192/192	R	192/192	100%
9	W7E26R	1:2	R	192/192	R	192/192	R	192/192	100%
10	N7H03	NR	NR	192/192	NR	192/192	NR	192/192	100%

The data above show that the ASI Evolution gives an objective and standardized interpretation of the test results with a high degree of reproducibility.

Cross Reactivity/Interfering Substances

A study was conducted to evaluate potential interference or cross reactivity from different disease conditions. Results are listed below:

Cross Reactivity/Interfering Substances

Specimen Category	Number of Samples	Expected Results	Results
ANA (+) Syphilis (-)	3	NR	NR
ASO (+) Syphilis (-)	2	NR	NR
CRP (+) Syphilis (-)	2	NR	NR
Infectious Mononucleosis* (+) Syphilis (-)	3	NR	NR
RF (+) Syphilis (-)	12	NR	NR
Rubella (+) Syphilis (-)	12	NR	NR
Lyme's (+) Syphilis (-)	12	NR	NR
HIV (+) Syphilis (-)	50	NR	NR
HIV (+) Syphilis (+)	24	R	R
Pregnancy (+) Syphilis (-)	250	NR	NR
Pregnancy (+) Syphilis (+)	30	R	R
Bilirubin 20 mg/dl	2	NR	NR
Hemoglobin 10 mg/ml	2	NR	NR
Triglycerides 1000mg/dl	2	NR	NR

*The positive infectious mononucleosis samples were heterophil antibody positive for the determination of disease state. EBV testing was not conducted.

The study showed no interference.

Carry-Over

A study was conducted to evaluate if contamination of a nonreactive sample due to carry-over from an adjacent reactive sample can occur.

- Testing was conducted at: Arlington Scientific, Inc.
- Testing was conducted using two different samples:
  - RPR reactive 1:64 tittered sample (high reactive) – Lot 06237
  - RPR nonreactive sample – Lot 06127
- The same samples were used for all testing.
- The same lot of carbon antigen was used – Lot CA7D24R

- Each test run was completed each day for five days by an operator with experience in performing the ASI RPR Card Test for Syphilis and operating the ASI Evolution.

- The test consisted of alternating 24 aliquots of the samples listed above in the sample rack and completing a run of 48 tests. Testing was performed on the same ASI Evolution.

The results of the testing are contained in table below:

Sample	Well Number	Expected	Results				
			ASiManager-AT #1				
		Date	08/11/17	08/14/17	08/15/17	08/16/17	08/17/17
06237	P1:A1	R	R	R	R	R	R
06127	P1:A2	NR	NR	NR	NR	NR	NR
06237	P1:A3	R	R	R	R	R	R
06127	P1:A4	NR	NR	NR	NR	NR	NR
06237	P1:A5	R	R	R	R	R	R
06127	P1:A6	NR	NR	NR	NR	NR	NR
06237	P1:A7	R	R	R	R	R	R
06127	P1:A8	NR	NR	NR	NR	NR	NR
06237	P1:B1	R	R	R	R	R	R
06127	P1:B2	NR	NR	NR	NR	NR	NR
06237	P1:B3	R	R	R	R	R	R
06127	P1:B4	NR	NR	NR	NR	NR	NR
06237	P1:B5	R	R	R	R	R	R
06127	P1:B6	NR	NR	NR	NR	NR	NR
06237	P1:B7	R	R	R	R	R	R
06127	P1:B8	NR	NR	NR	NR	NR	NR
06237	P1:C1	R	R	R	R	R	R
06127	P1:C2	NR	NR	NR	NR	NR	NR
06237	P1:C3	R	R	R	R	R	R
06127	P1:C4	NR	NR	NR	NR	NR	NR
06237	P1:C5	R	R	R	R	R	R
06127	P1:C6	NR	NR	NR	NR	NR	NR
06237	P1:C7	R	R	R	R	R	R
06127	P1:C8	NR	NR	NR	NR	NR	NR
06237	P1:D1	R	R	R	R	R	R
06127	P1:D2	NR	NR	NR	NR	NR	NR
06237	P1:D3	R	R	R	R	R	R
06127	P1:D4	NR	NR	NR	NR	NR	NR
06237	P1:D5	R	R	R	R	R	R
06127	P1:D6	NR	NR	NR	NR	NR	NR
06237	P1:D7	R	R	R	R	R	R
06127	P1:D8	NR	NR	NR	NR	NR	NR
06237	P1:E1	R	R	R	R	R	R
06127	P1:E2	NR	NR	NR	NR	NR	NR
06237	P1:E3	R	R	R	R	R	R

06127	P1:E4	NR	NR	NR	NR	NR	NR
06237	P1:E5	R	R	R	R	R	R
06127	P1:E6	NR	NR	NR	NR	NR	NR
06237	P1:E7	R	R	R	R	R	R
06127	P1:E8	NR	NR	NR	NR	NR	NR
06237	P1:F1	R	R	R	R	R	R
06127	P1:F2	NR	NR	NR	NR	NR	NR
06237	P1:F3	R	R	R	R	R	R
06127	P1:F4	NR	NR	NR	NR	NR	NR
06237	P1:F5	R	R	R	R	R	R
06127	P1:F6	NR	NR	NR	NR	NR	NR
06237	P1:F7	R	R	R	R	R	R
06127	P1:F8	NR	NR	NR	NR	NR	NR

These data demonstrate that all testing results were as expected and there was no evidence of contamination or carry-over.

A comparison of the digital interpretation of the results from the ASI Evolution using the original interpretation algorithm (K173376, BK170114, and K182391) to establish substantial equivalence to the interpretation made by the ASI Evolution using the new interpretation algorithm was conducted.

The ASI Evolution was evaluated for equivalence, in its pattern of reactivity using a total of 1,762 individual retrospective samples, with identifiers removed, that had been collected from different Departments of Public Health Labs and Blood Banks. Reactive, Weak Reactive and Nonreactive controls were run on each day of testing.

Retrospective Serum Sample Testing – 872 Samples

		ASI Evolution New Algorithm		
		Reactive	**Nonreactive**	**Total**
ASI Evolution Original Algorithm	**Reactive**	91	0	91
	Nonreactive	6	775	781
	Total	97	775	872

Note: The six discordant results were investigated and tested with a treponemal test and found to be reactive.

Serum positive agreement is calculated as:

91/(91 + 0) = 100%
95% CI = 96.03% - 100%

Serum negative agreement is calculated as:

775/(775 + 6) = 99.23%
95% CI = 98.34% - 99.72%

Serum samples were from both SST and Red Top tubes.

Retrospective Serum Sample Testing – 890 Samples

		ASI Evolution New Algorithm		
		Reactive	Nonreactive	Total
ASI Evolution Original Algorithm	Reactive	119	5	124
	Nonreactive	1	765	766
	Total	120	770	890

Note: The six discordant results were investigated and the sample that was called reactive by the new algorithm and nonreactive by the original algorithm was tested with a treponemal test and found to be nonreactive. The five samples that were called nonreactive by the new algorithm and reactive by the original algorithm had bubbles or artifacts in the test well.

Total Plasma positive agreement is calculated as:
119/(119 + 5) = 95.97%
95% CI = 90.84% - 98.68%

Sodium Citrate positive agreement is calculated as:
55/(55 + 4) = 93.22%
95% CI = 83.54% - 98.12%

EDTA positive agreement is calculated as:
64/(64 + 1) = 98.46%
95% CI = 91.72% - 99.96%

Total Plasma negative agreement is calculated as:
765/(765 + 1) = 99.87%
95% CI = 99.27% - 100%

Sodium Citrate negative agreement is calculated as:
465/(465 + 1) = 99.79%
95% CI = 98.81% - 99.99%

EDTA negative agreement is calculated as:
300/(300 + 0) = 100%
95% CI = 98.78% - 100%

The positive and negative percent agreement for the two algorithms demonstrate that they have a very similar performance.

Reproducibility
Reproducibility testing was conducted. The testing consisted of:

- Testing seven (7) samples
    - 2 – RPR nonreactive samples
    - 2 – RPR reactive 1:2 titered samples
    - 1 – RPR reactive 1:4 titered sample
    - 1 – RPR reactive 1:8 titered sample
    - 1 – RPR reactive 1:16 titered sample
- Each sample was run in duplicate within the panel.
- Each sample was tested each day for five non-consecutive days by an operator with experience in performing the ASI Automated RPR Test for Syphilis
- Each sample was tested a second time on each of the days referenced above separated by approximately 2 hours.

Reproducibility Results

RPR (Rapid Plasma Reagin)				
Sample	**Sample #**	**N**	**Expected Result**	**95% Confidence Interval**
RPR nonreactive	10159A	60	100% (60/60)	94.04 - 100
RPR nonreactive	06127	60	100% (60/60)	94.04 - 100
RPR reactive 1:2	10159D	60	100% (60/60)	94.04 - 100
RPR reactive 1:2	W9P19R	60	100% (60/60)	94.04 - 100
RPR reactive 1:4	10159C	60	100% (60/60)	94.04 - 100
RPR reactive 1:8	10159E	60	100% (60/60)	94.04 - 100
RPR reactive 1:16	R0B03R	60	100% (60/60)	94.04 - 100

The data show a very high degree of reproducibility.

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