For in vitro diagnostic use

Catalog Number	***Kit Size***
900480D	**480 Tests**
9004800D	**4800 Tests**

℞Only

CPT Code: 86592 (Screening) and 86593 (Diagnostic)

1 INTENDED USE:

The ASI Automated RPR (rapid plasma reagin) Test for Syphilis, for use on the ASI Evolution Automated Analyzer, is a qualitative and semiquantitative flocculation test for the detection of nontreponemal antibodies in human serum and plasma to aid in the diagnosis of syphilis. All reactive RPR test samples should be further tested with a treponemal test to determine serological evidence of syphilis infection. The test is intended to be used for *in vitro* diagnostic testing.

The ASI Automated RPR 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]. The traditional testing approach for evidence of syphilis consist of a two-tier approach, where the patient is first screened with a non-treponemal test and, if reactive, additional testing is performed with a treponemal assay. Diagnosis of syphilis is made when at least two tests for syphilis produce reactive results and in conjunction with clinical information.

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. It is intended to be acquired, possessed, and used only by health care professionals. The ASI Evolution analyzer, in conjunction with the ASI Automated RPR Test for Syphilis is intended to be used for *in vitro* diagnostic testing.

3 PRINCIPLE OF THE PROCEDURE:

The **ASI Automated RPR Test for Syphilis** 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 and internal camera an image processing algorithm to read the RPR agglutination reaction and report a reactive or non-reactive result. The analyser is also capable of performing end-point titers.

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 stabilizers.

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

4.3 Reagents have two-year expiration dating from date of manufacture. The specific expiration date is located on the label on the vial. Open and closed dating are the same.

4.4 On-board storage stability is 8 hours. Return reagents to 2-8° C when not in use.

Materials Provided:

	480 Tests	**4800 Tests**
RPR CARBON ANTIGEN	23 ml	23 ml x 10
Microwell Plates (48 well)	10	100

Materials Sold Separately:

	480 Tests	**4800 Tests**
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

- Titration Diluent – 20 ml - for use when doing end-point titrations

5 WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

5.1 **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.

5.2 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.

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

6.3 Do not interchange components from this kit with those of a different manufacturer.

7 STORAGE INSTRUCTIONS

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

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 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.

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. 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].

9.4 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.

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 TEST PROCEDURE – Qualitative/Semiquantitative

10.1 Allow all reagents and samples to come to room temperature.

10.2 Create or select a work list.

10.3 Load samples as work list is created.

10.4 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.

10.5 Select test to perform: "R/NR" for qualitative testing or "TITERS" for Semiquantitative testing.

10.6 Name work list.

10.7 Close Cover

10.8 Press start.

10.9 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.

Note: The qualitative procedure is used to determine if a specimen is reactive or nonreactive. The semiquantitative procedure is performed on reactive samples to determine the end-point titer for use in following effectiveness of treatment.

11 Interpretation of Results

Qualitative Results:
The instrument will give the result of the interpretation of the test well reaction as either nonreactive or reactive.

Reactive nontreponemal results alone are not diagnostic of syphilis and should always be followed up with a treponemal test. Serologic test results should be interpreted in conjunction with clinical findings.

Semiquantitative Results:
The instrument will give an end-point titer result for the test, (such as, 1:2, 1:4, etc.), which is the last well that gave a reactive result. Maximum end-point titer of 1:2048.

Nontreponemal test antibody titers might correlate with disease activity and are used to follow treatment response.

12 QUALITY CONTROL

12.1 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.

13 LIMITATIONS OF THE PROCEDURE

13.1 The device should not be used for syphilis testing with the Reverse Testing Algorithm (when treponemal testing is conducted first). This device should only be used when RPR testing is conducted before any follow up treponemal assays.

13.2 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.

13.3 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.

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

13.5 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].

13.6 The cover of the ASI Evolution should be closed while tests are being performed to avoid glare from outside lighting sources.

13.7 Reactive RPR test samples should be followed up with treponemal antibody testing as recommended in the Manual of Tests for Syphilis[2, 6].

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

13.9 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.

14 PERFORMANCE CHARACTERISTICS

Clinical Performance

The **ASI Automated RPR Test for Syphilis** on the ASI Evolution was evaluated for equivalence in its pattern of reactivity against the ASI RPR Card Test for Syphilis on the ASiManager-AT. A total of 1,068 individual prospective serum samples, with identifiers removed, were collected at two different Departments of Public Health Labs and tested by the **ASI Automated RPR Test for Syphilis** on the ASI Evolution in comparison with the ASI RPR Card Test for Syphilis on the ASiManager-AT at each of the facilities. Reactive, Weak Reactive and Nonreactive controls were run on each day of testing. The results were as follows:

Prospective Sample Testing – 1,068 Samples

		ASI RPR Card Test for Syphilis on the ASiManager-AT Results	
		Reactive	Nonreactive
ASI Automated RPR Test for Syphilis on the ASI Evolution Results	Reactive	114	1
	Nonreactive	1	952

Using the data from the prospective performance results above, the positive percent agreement of the **ASI Automated RPR Test for Syphilis on the ASI Evolution** with the ASI RPR Card Test on the ASiManager was:

PPA = 99.13% (114/115); 95% CI: (95.25% - 99.98%)

Using the data from the prospective performance results above, the negative percent agreement of the **ASI Automated RPR Test for Syphilis on the ASI Evolution** with the ASI RPR Card Test on the ASiManager was:

NPA = 99.90% (952/953); 95% CI: (99.42% - 100.00%)

A total of 1,013 individual retrospective samples (10 serum, 1003 plasma), with identifiers removed, were collected from various reference labs and serum and sodium citrate plasma vendors from across the United States and tested by the **ASI Automated RPR Test for Syphilis** on the ASI Evolution in comparison with the ASI RPR Card Test for Syphilis on the ASiManager-AT Results. Reactive, Weak Reactive and Nonreactive controls were run on each day of testing.

Retrospective Serum Sample Testing – 10 Samples

		ASI RPR Card Test for Syphilis on the ASiManager-AT Results	
		Reactive	Nonreactive
ASI Automated RPR Test for Syphilis on the ASI Evolution Results	Reactive	7	0
	Nonreactive	0	3

Serum positive percent agreement was:

PPA = 100.00% (7/7); 95% CI: (59.04% - 100.00%)

Serum negative percent agreement was:

NPA = 100.00% (3/3); 95% CI: (29.24% - 100.00%)

Retrospective Plasma Sample Testing – 1,003 Samples

		ASI RPR Card Test for Syphilis on the ASiManager-AT Results	
		Reactive	Nonreactive
ASI Automated RPR Test for Syphilis on the ASI Evolution Results	Reactive	10	0
	Nonreactive	0	993

Sodium Citrate plasma positive percent agreement was:

PPA = 100.00% (10/10); 95% CI: (69.15% - 100.00%)

Sodium Citrate plasma negative percent agreement was:

NPA = 100.00% (993/993); 95% CI: (99.63% - 100.00%)

Distribution of Samples at Testing Sites

Site	Prospective random samples			Retrospective samples			Grand Total
				Serum & Plasma			
	Plasma	Serum	Total	Known infected	Known Uninfected	Total	
a	0	567	567	0	0	0	567
b	0	501	501	0	0	0	501
c	0	0	0	17	996	1,013	1,013
total	0	1,068	**1,068**	17	996	**1,013**	**2,081**

Characterized Specimen Testing on ASI Automated RPR Test for Syphilis on the **ASI Evolution**

- Testing was conducted at Arlington Scientific, Inc. – Site C
- Carbon antigen lot used – Lot CA5K01RBA
- Each sample was tested by an operator with experience in performing the ASI Automated RPR Test for Syphilis and operating the ASI Evolution.
- ASI Evolution unit 5800-0102 (#1) used for testing
- Expected results are based on known clinical diagnosis of syphilis
- All samples were serum

The results of the testing are contained in the table below:

Characterized Specimen Testing

Clinical Syphilis Diagnosis	**No. Reactive**	**No. Nonreactive**	**% Agreement**	**95% CI**
Primary Treated	25	0	100%	86.28 – 100%
Primary Untreated	18	0	100%	81.47 – 100%
Secondary Treated	25	0	100%	86.28 – 100%
Secondary Untreated	25	0	100%	86.28 – 100%
Latent Treated	25	0	100%	86.28 – 100%
Latent Untreated	25	0	100%	86.28 – 100%

Performance with Samples from Pregnant Women:

Samples were collected from 250 pregnant women and tested on the **ASI Automated RPR Test for Syphilis** on the ASI Evolution for reactivity. These samples were nonreactive for nontreponemal antibodies when tested using the ASI RPR Card Test for Syphilis on the ASiManager-AT. The age of these women ranged from 15 to 41 years old (with a median age of 28 years old). All samples were nonreactive when tested with the **ASI Automated RPR Test for Syphilis** on the ASI Evolution and the comparator. Samples collected from 30 pregnant women that had been diagnosed as having syphilis and, were reactive by the comparator nontreponemal test, were tested with the **ASI Automated RPR Test for Syphilis** on the ASI Evolution for reactivity. The women ranged in age from 18 to 40 years old (with a median age of 28 years old). All samples were reactive with the **ASI Automated RPR Test for Syphilis**. All the samples were serum.

Pregnant Women Testing

		ASI RPR Card Test for Syphilis on the ASiManager-AT Results	
		Reactive	Nonreactive
ASI Automated RPR Test for Syphilis on the ASI Evolution Results	Reactive	30	0
	Nonreactive	0	250

There was 100% agreement in results between the **ASI Automated RPR Test and the ASI RPR Card Test on the ASiManager-AT** for all samples from pregnant women.

Analytical Performance

Precision

The precision of the ASI Automated RPR Test for Syphilis on the **ASI Evolution** was evaluated using 10 samples.

The testing requirements were as follows:

1. All qualitative testing was conducted according to the procedure listed in the package insert.
2. Each sample was tested 192 times. The number of replicates were chosen since the instrument can hold four 48-well plates for a total of 192 wells. The 192 samples will test each well position.

Of the 10 samples:

3 were nonreactive
4- RPR reactive 1:1 titered samples
1- RPR reactive 1:2 titered sample
1- RPR reactive 1:8 titered sample
1- RPR reactive 1:256 titered sample
Note: All end-point titers were determined by the manual method.

Each of the 10 samples was repeated 192 times to evaluate the reactivity of the **ASI Automated RPR Test for Syphilis on** the **ASI Evolution**. An aliquot of the same sample was dispensed into 192 tubes. All 192 tubes were placed into the ASI Evolution and the run was performed. In this manner, all 192 wells were tested with the same sample to show well to well and plate to plate repeatability using four test plates.

Precision Testing

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

Note: All expected end-point titers were originally determined by manual RPR titer

The data above demonstrated 100% agreement with expected results for all samples tested.

Reproducibility

Reproducibility testing was conducted at three sites. The testing consisted of:

- Testing seven (7) samples
  - 2 - RPR nonreactive samples
  - 1 – RPR reactive 1:2 titered samples
  - 1 – RPR reactive 1:4 titered sample
  - 2 - 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 and operating the ASI Evolution.
- Each sample was tested a second time on each of the days referenced above separated by approximately 2 hours.
- 3 lots were tested each day.
- Testing at each site was performed on the same ASI Evolution instrument.

Reproducibility Testing Table 1

RPR			Site 1		Site 2		Site 3		Total	
Sample RPR Status	***Sample ID***	***N***	***% Agreement with Expected Result***	***95% Confidence Interval***	***% Agreement with Expected Result***	***95% Confidence Interval***	***% Agreement with Expected Result***	***95% Confidence Interval***	***% Agreement with Expected Result***	***95% Confidence Interval***
RPR nonreactive	02287	180	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (180/180)	97.91 - 100
RPR nonreactive	N6K14	180	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (180/180)	97.91 - 100
RPR reactive 1:2	05225B	180	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (180/180)	97.91 - 100
RPR reactive 1:4	07035	180	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (180/180)	97.91 - 100
RPR reactive 1:8	05225A	180	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (180/180)	97.91 - 100
RPR reactive 1:8	R7C21R	180	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (180/180)	97.91 - 100
RPR reactive 1:16	07117	180	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (60/60)	93.98 - 100	100% (180/180)	97.91 - 100

The data above demonstrated 100% agreement with expected results for all samples tested.

Another reproducibility study was conducted at the three sites. All qualitative testing was conducted according to the procedure listed in the package insert. The testing consisted of:

- Testing 448 samples
  - 48 - RPR nonreactive samples
  - 400 – RPR reactive samples
- The same samples were tested at all three sites
- All samples were retrospective serum.
- Samples, with identifiers removed, were collected from various reference labs and serum and plasma vendors from across the United States.
- Testing at each site was performed on the same ASI Evolution.
- Results were as follows:

Reproducibility Testing Table 2

RPR		Site 1		Site 2		Site 3		Total	
Sample	***N***	***% Agreement with Expected Result***	***95% Confidence Interval***	***% Agreement with Expected Result***	***95% Confidence Interval***	***% Agreement with Expected Result***	***95% Confidence Interval***	***% Agreement with Expected Result***	***95% Confidence Interval***
RPR nonreactive	48	100% (48/48)	92.60 - 100	100% (48/48)	92.60 - 100	100% (48/48)	92.60 - 100	100% (144/144)	97.47 - 100
RPR reactive	400	100% (400/400)	99.08 - 100	100% (400/400)	99.08 - 100	100% (400/400)	99.08 - 100	100% (1200/1200)	99.69 - 100

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 Result	# of samples with expected result/# of samples tests
ANA (+) Syphilis (-)	3	NR	3/3
ASO (+) Syphilis (-)	2	NR	2/2
CRP (+) Syphilis (-)	2	NR	2/2
Infectious Mono (+) Syphilis (-)[1]	3	NR	3/3
RF (+) Syphilis (-)	12	NR	12/12
Rubella (+) Syphilis (-)	12	NR	12/12
Lyme's (+) Syphilis (-)	12	NR	12/12
HIV (+) Syphilis (-)	50	NR	50/50
HIV (+) Syphilis (+)	24	R	24/24
Pregnancy (+) Syphilis (-)	250	NR	250/250
Pregnancy (+) Syphilis (+)	30	R	30/30
Bilirubin 20 mg/dl	2	NR	2/2
Hemoglobin 10 mg/ml	2	NR	2/2
Triglycerides 1000mg/dl	2	NR	2/2

[1] The positive infectious mono samples were determined by heterophile testing; 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 titered sample (high reactive) – 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 Automated RPR Test for Syphilis and operating the ASI Evolution.
- A test run 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.

Carry-Over Testing

		Expected Results	
		Reactive	Nonreactive
ASI Evolution Results	Reactive	24	0
	Nonreactive	0	24

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

End-Point Titration Testing

A randomized and blinded panel of 10 human serum samples with known reagin antibody endpoint titers, as determined by the ASI RPR Card Test for Syphilis on the ASiManager-AT, were tested with the ASI Automated RPR Test for Syphilis on the ASI Evolution using the semiquantitative test procedure. The reactive samples had titers ranging from 1:1 to 1:256. Each sample panel member was tested a minimum of 80 times on at least five different days by a single operator using a single ASI Evolution instrument. All nonreactive samples must yield nonreactive test results, while all reactive samples must yield results that are within one dilution above or below the known titer. The results of the semiquantitative analysis are shown below.

Endpoint Titer Results												
Sample ID and RPR Reactivity Status	Nonreactive	Neat (1:1)	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	% Agreement within +/- 1 titer (95% C.I.)
06127 (Nonreactive)	80	0	0	0	0	0	0	0	0	0	0	100% (95.49% - 100%)
N8E23 (Nonreactive)	80	0	0	0	0	0	0	0	0	0	0	100% (95.49% - 100%)
W6A16R (1:2)	0	7	69	4	0	0	0	0	0	0	0	100% (95.49% - 100%)
W8B01R (1:2)	0	0	75	5	0	0	0	0	0	0	0	100% (95.49% - 100%)
R7F01R (1:8)	0	0	0	0	76	4	0	0	0	0	0	100% (95.49% - 100%)
R8B01R (1:8)	0	0	0	32	48	0	0	0	0	0	0	100% (95.49% - 100%)
07117 (1:16)	0	0	0	0	5	75	0	0	0	0	0	100% (95.49% - 100%)
08188 (1:64)	0	0	0	0	0	0	0	87	1	0	0	100% (95.89% - 100%)
07098 (1:128)	0	0	0	0	0	0	0	5	82	1	0	100% (95.89% - 100%)
08296 (1:256)	0	0	0	0	0	0	0	0	21	65	4	100% (95.98% - 100%)

All nonreactive samples were nonreactive, and all remaining samples were reactive within one dilution of the known titer for an overall percent agreement of 100%.

Another study was conducted where nine samples were tested in eight replicates on 17 different days. Not all samples were tested on the same day. Each sample set of eight replicates was tested 10 times giving a total of 80 data points for each sample. An acceptable result is within +/- 1 titer of the expected result. Nonreactive samples must be nonreactive. The results of the semiquantitative analysis for the nine samples are shown below:

Titration Sample Testing

Endpoint Titer Results												
Sample ID and RPR Reactivity Status	Nonreactive	Neat (1:1)	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	% Agreement within +/- 1 titer (95% C.I.)
06127 (Nonreactive)	80	0	0	0	0	0	0	0	0	0	0	100% (95.49% - 100%)
N8E23 (Nonreactive)	80	0	0	0	0	0	0	0	0	0	0	100% (95.49% - 100%)
W6A16R (1:2)	0	6	70	4	0	0	0	0	0	0	0	100% (95.49% - 100%)
W8B01R (1:2)	0	0	76	4	0	0	0	0	0	0	0	100% (95.49% - 100%)
R7F01R (1:8)	0	0	0	0	76	4	0	0	0	0	0	100% (95.49% - 100%)
R8B01R (1:8)	0	0	0	15	65	0	0	0	0	0	0	100% (95.49% - 100%)
08188 (1:64)	0	0	0	0	0	0	0	79	1	0	0	100% (95.89% - 100%)
07098 (1:128)	0	0	0	0	0	0	0	3	76	1	0	100% (95.89% - 100%)
08296 (1:256)	0	0	0	0	0	0	0	0	10	66	4	100% (95.98% - 100%)

All titration samples were within the +/- one titer.

The ASI Evolution was evaluated for equivalence in its pattern of reactivity against the ASiManager-AT. A total of 3,757 tests (2,861 individual samples) were conducted by the ASI Evolution compared with the ASiManager-AT. The same panel of 448 samples was tested at three sites: all samples 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.

Distribution of Samples to the Sites

Site	Prospective random samples		Retrospective 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**	**1,200**	**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 results of testing serum samples on the ASI Evolution and the ASiManager-AT

		ASiManager-AT Results	
		Reactive	Nonreactive
ASI Evolution Results	Reactive	416	0
	Nonreactive	4	438

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

PPA = 99.05% (416/420); 95% CI: (97.58% - 99.74%)
NPA = 100.00% (438/438); 95% CI: (99.16% - 100.00%)

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

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

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

PPA = 100.00% (413/413); 95% CI: (99.11% - 100.00%)

NPA = 100.00% (1590/1590); 95% CI: (99.77% - 100.00%)

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 below.

Instrument Reproducibility

	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%

Note: All expected end-point titers were originally determined by manual RPR titer

The data showed 100% agreement with expected results.

Software Modification

There was a modification to the interpretation algorithm to appropriately categorize sample results. The following performance data was generated with the modified software.

A comparison of the digital interpretation of the results from the ASI Evolution using the original interpretation algorithm 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 (872 serum and 890 plasma), 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. The results of the testing are shown in the following two tables.

Retrospective Serum Sample Testing – 872 Samples

		ASI Evolution Original Algorithm	
		Reactive	Nonreactive
ASI Evolution New Algorithm	Reactive	91	6
	Nonreactive	0	775

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

Serum positive percent agreement is calculated as:

PPA = 100.00% (91/91); 95% CI: (96.03% - 100.00%)

Serum negative percent agreement is calculated as:

NPA = 99.23% (775/781); 95% CI: (98.34 – 99.72%)

Serum samples were from both SST and Red Top tubes.

Retrospective Plasma Sample Testing – 890 Samples

		ASI Evolution Original Algorithm	
		Reactive	Nonreactive
ASI Evolution New Algorithm	Reactive	119	1
	Nonreactive	5	765

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 nonreactive by the new algorithm were shown to have bubbles or artifacts in the test well and were incorrectly called “reactive” by the original algorithm.

Total Plasma positive percent agreement is calculated as:

PPA = 95.97% (119/124); 95% CI: (90.84% - 98.68%)

Sodium Citrate positive percent agreement is calculated as:

PPA = 93.22% (55/59); 95% CI: (83.54% - 98.12%)

EDTA positive percent agreement is calculated as:

PPA = 98.46% (64/65); 95% CI: (91.72% - 99.96%)

Total Plasma negative percent agreement is calculated as:

NPA = 99.87% (765/766); 95% CI: (99.27% - 100.00%)

Sodium Citrate negative percent agreement is calculated as:

NPA = 99.79% (465/466); 95% CI: (98.81% - 99.99%)

EDTA negative percent agreement is calculated as:

NPA = 100.00% (300/300); 95% CI: (98.78% - 100.00%)

Pregnant Women Testing

		ASI RPR Card Test for Syphilis on the ASiManager-AT Result	
		Reactive	Nonreactive
ASI Automated RPR Test for Syphilis on the ASI Evolution Result	Reactive	30	0
	Nonreactive	0	250

The data from testing pregnant women demonstrated 100% agreement between the results from the ASI Automated RPR Test for Syphilis on the ASI Evolution and the results from the ASI RPR Card Test for Syphilis on the ASiManager-AT. The samples consisted of 121 first trimester, 98 second trimester and 61 third trimester.

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 Testing

RPR				
Sample RPR Status	***Sample ID***	***N***	***% Agreement with 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

Note: All expected end-point titers were originally determined by manual RPR titer

The data shows a very high degree of reproducibility.

End-point Titer Testing

A total of nine samples with identifiers removed were tested to determine patterns of reactivity using the semiquantitative test procedure on the ASI Evolution with the new interpretation algorithm with the reagents of the ASI Automated RPR Test for Syphilis. There were no errors with the instrument during the testing.

The nine samples were made up of prospective control serum and serum samples of known reactivity. All samples had been tested by the manual RPR interpretation method prior to testing. The expected results were established by this testing. These specimens were tested with ASI carbon antigen (CA9P02RRD). The samples were as follows:

Sample ID	Sample Type	Titer (manual)
06127	Normal Human Serum	Nonreactive (NR)
10159A	Nonreactive Control	Nonreactive (NR)
10159E	Human Serum	Reactive (1:8)
10159D	Human Serum	Reactive (1:2)
W9P19R	Weak Reactive Control	Reactive (1:2)
R0B03R	Reactive Control	Reactive (1:8)
01140	Human Serum	Reactive (1:64)
10159	Human Serum	Reactive (1:128)
10189C	Human Serum	Reactive (1:256)

Note: All expected end-point titers were originally determined by manual RPR titer

The nine samples were tested in eight replicates on ten different days. Not all samples were tested on the same day. Each sample set of eight replicates was tested ten times giving a total of 80 data points for each sample. An acceptable result is within +/- 1 titer of the expected result. Nonreactive samples must be nonreactive. The results of the semiquantitative analysis samples are shown in tables below:

Titration Sample Testing

RPR Endpoint Manual Testing			RPR Endpoint Titer New Algorithm								
	Nonreactive	1:1 (Neat)	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Nonreactive	80										
Nonreactive	80										
1:2			43	37							
1:2			58	22							
1:8					65	15					
1:8				20	55	5					
1:64							17	46	17		
1:128								26	53	1	
1:256									35	40	5

15 **REFERENCES**

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