

Title: ASI AUTOMATED RPR TEST FOR SYPHILIS FOR USE ON THE ASI EVOLUTION (BLOOD BANK)		Doc#: 6004-2800ABB CLSI
Effective Date: 03/17/2021	Supersedes Revision/Date: 03/17/2021 (1)	Revision: 03/17/2021 (1)

For in vitro diagnostic use

<u>Catalog Number</u>	<u>Kit Size</u>
900480ABB (Blood Bank)	480 Tests
9004800ABB (Blood Bank)	4800 Tests

Rx Only

CPT Code: 86592 (Screening)

1 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 and for cadaveric (non-heart beating) donor specimens for tissue donation. This test is not intended for diagnostic use.

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 antigen¹, and anti-nontreponemal antibodies (reagin) that can be detected by RPR antigen².

3 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 an image processing algorithm to read the RPR agglutination reaction and report a reactive or non-reactive result.

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.

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.

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- 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 it².
- 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 testing².
- 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 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

Also available without controls

11 TEST PROCEDURE – Qualitative

- 11.1 Create or select a work list.
- 11.2 Load samples as work list is created.
- 11.3 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.
- 11.4 Select test to perform. R/NR for qualitative testing.
- 11.5 Name work list.
- 11.6 Close Cover
- 11.7 Press start.
- 11.8 Dispose of used microtiter plates in accordance with federal (40 CFR 261.3), state, local or Good Laboratory Practice requirements.

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See Operator's Manual for complete instructions.

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 Prozone reactions can occur in patients with secondary syphilis³. False negative nontreponemal test results, arising from prozone, can also be seen in incubating primary and in late syphilis². 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.
- 13.2 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.
- 13.3 Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test².
- 13.4 The cover of the ASI Evolution should be closed while tests are being performed to avoid glare from outside lighting sources.
- 13.5 Temperature of the reagents and samples is crucial to test outcome; it should be between 20-30°C.
- 13.6 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.

14 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		Retrospective samples ³				A panel of known reactivity ³		Total
	Plasma ¹	Serum ²	Plasma		Serum		Serum		
			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.

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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.

$$\text{PPA} = 416/420 = 99.05\% \text{ 95\% CI: (97.58\%, 99.74\%)}$$

$$\text{NPA} = 438/438 = 100\% \text{ 95\% CI: (99.16\%, 100\%)}$$

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): $400 + 7 = 407$.

$$\text{Specificity} = 391/391 = 100\% \text{ 95\% CI: (99.06\%, 100\%)}$$

$$\text{Sensitivity} = 407/407 = 100\% \text{ 95\% CI: (99.10\%, 100\%)}$$

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.

$$\text{PPA} = 413/413 = 100\% \text{ 95\% CI: (99.11\%, 100\%)}$$

$$\text{NPA} = 1590/1590 = 100\% \text{ 95\% CI: (99.77\%, 100\%)}$$

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).

$$\text{Specificity} = 597/597 = 100\% \text{ 95\% CI: (99.38\%, 100\%)}$$

$$\text{Sensitivity} = 410/410 = 100\% \text{ 95\% CI: (99.10\%, 100\%)}$$

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.

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Table 4

	Sample	Expected	Results						% Agreement
			Sample ID	Titer	Evolution 1		Evolution 2		
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 Result	Result
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.

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- 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

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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:

$$91/(91 + 0) = 100\%$$

$$95\% \text{ CI} = 96.03\% - 100\%$$

Serum negative agreement is calculated as:

$$775/(775 + 6) = 99.23\%$$

$$95\% \text{ CI} = 98.34\% - 99.72\%$$

Serum samples were from both SST and Red Top tubes.

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Retrospective Plasma 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:

$$119/(119 + 5) = 95.97\%$$

$$95\% \text{ CI} = 90.84\% - 98.68\%$$

Sodium Citrate positive agreement is calculated as:

$$55/(55 + 4) = 93.22\%$$

$$95\% \text{ CI} = 83.54\% - 98.12\%$$

EDTA positive agreement is calculated as:

$$64/(64 + 1) = 98.46\%$$

$$95\% \text{ CI} = 91.72\% - 99.96\%$$

Total Plasma negative agreement is calculated as:

$$765/(765 + 1) = 99.87\%$$

$$95\% \text{ CI} = 99.27\% - 100\%$$

Sodium Citrate negative agreement is calculated as:

$$465/(465 + 1) = 99.79\%$$

$$95\% \text{ CI} = 98.81\% - 99.99\%$$

EDTA negative agreement is calculated as:

$$300/(300 + 0) = 100\%$$

$$95\% \text{ CI} = 98.78\% - 100\%$$

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

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- 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.

Cadaveric Donor Specimens:

Testing was done comparing cadaveric donor samples and living tissue donor samples. The results were as follows:

A total of 164 serum samples, collected in SST (90) and red top tubes (74), with identifiers removed were tested to determine patterns of reactivity using the qualitative RPR test with the ASI Automated RPR Test procedure on the ASI Evolution. All the cadaveric specimens were collected within 24 hours of death. All specimens were shipped with ice packs by overnight delivery to ASI. Each sample was evaluated for the degree of hemolysis. The amount of hemolysis in the samples ranged from none to a heavy degree.

The results of the qualitative analysis of the serum samples collected in red top tubes (prior to and after spiking with reactive sample) are shown below:

Serum Sample (Red Top) Testing - 74 Samples

Known Nonreactive

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	0	0	0
	Nonreactive	0	74	74
	Total	0	74	74

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Spiked Samples

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	74	0	74
	Nonreactive	0	0	0
	Total	74	0	74

Review and analysis of the data for the samples give a percent agreement of 100% for nonreactive samples and 100% for reactive samples.

The results of the qualitative analysis of the serum samples collected in SST tubes (prior to and after spiking with reactive sample) are shown below:

Serum Sample (SST) Testing - 90 Samples

Known Nonreactive

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	0	0	0
	Nonreactive	0	90	90
	Total	0	90	90

Spiked Samples

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	90	0	90
	Nonreactive	0	0	0
	Total	90	0	90

Review and analysis of the data for the samples give a percent agreement of 100% for nonreactive samples and 100% for reactive samples.

A total of 84 EDTA plasma samples, with identifiers removed were tested to determine patterns of reactivity using the qualitative RPR test with the ASI Automated RPR Test procedure on the ASI Evolution. All the cadaveric specimens were collected within 24 hours of death. All specimens were shipped with ice packs by overnight delivery to ASI. Each sample was evaluated for the degree of hemolysis. The amount of hemolysis in the samples ranged from none to a heavy degree.

The results of the qualitative analysis of the samples collected from EDTA plasma tubes (prior to and after spiking with reactive sample) are shown in below:

EDTA Plasma Sample Testing - 84 Samples

Known Nonreactive

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	0	0	0
	Nonreactive	0	84	84
	Total	0	84	84

Spiked Samples

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ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	84	0	84
	Nonreactive	0	0	0
	Total	84	0	84

Review and analysis of the data for the samples give a percent agreement of 100% for nonreactive samples and 100% for reactive samples.

Living Donor Specimens:

A total of 126 serum samples, collected in SST (51) and red top (75) tubes, with identifiers removed were tested to determine patterns of reactivity using the qualitative RPR test with the ASI Automated RPR Test procedure on the ASI Evolution.

The results of the qualitative analysis of the serum samples collected in red top tubes (prior to and after spiking with reactive sample) are shown in below:

Serum Sample (Red Top) Testing - 75 Samples

Known Nonreactive

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	0	0	0
	Nonreactive	0	75	75
	Total	0	75	75

Spiked Samples

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	75	0	75
	Nonreactive	0	0	0
	Total	75	0	75

Review and analysis of the data for the samples give a percent agreement of 100% for nonreactive samples and 100% for reactive samples.

The results of the qualitative analysis of the serum samples collected in SST tubes (prior to and after spiking with reactive sample) are shown in below:

Serum Sample (SST) Testing - 51 Samples

Known Nonreactive

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	0	0	0
	Nonreactive	0	51	51
	Total	0	51	51

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Spiked Samples

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	51	0	51
	Nonreactive	0	0	0
	Total	51	0	51

Review and analysis of the data for the samples give a percent agreement of 100% for nonreactive samples and 100% for reactive samples.

A total of 76 EDTA plasma samples, with identifiers removed were tested to determine patterns of reactivity using the qualitative RPR test with the ASI Automated RPR Test procedure on the ASI Evolution. The results of the qualitative analysis of the samples collected from EDTA plasma tubes (prior to and after spiking with reactive sample) are shown in below:

EDTA Plasma Sample Testing - 76 Samples

Known Nonreactive

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	0	0	0
	Nonreactive	0	76	76
	Total	0	76	76

Spiked Samples

ASI Evolution Results				
		Reactive	Nonreactive	Total
ASiManager-AT Results	Reactive	76	0	76
	Nonreactive	0	0	0
	Total	76	0	76

Review and analysis of the data for the samples give a percent agreement of 100% for nonreactive samples and 100% for reactive samples.

ASI Evolution Performance Characteristics

Positive Agreement

Using the data from the composite performance results above, the positive agreement of the **ASI Evolution** can be calculated:

Cadaveric Donor Serum (Red Top) $74/(74+0) = 100\%$ 95% CI = 95.14% - 100%	Cadaveric Donor Serum (SST) $90/(90+0) = 100\%$ 95% CI = 95.98% - 100%	Cadaveric Donor Plasma (EDTA) $84/(84+0) = 100\%$ 95% CI = 95.70% - 100%
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Living Donor Serum (Red Top) $75/(75+0) = 100\%$ 95% CI = 95.20% - 100%	Living Donor Serum (SST) $51/(51+0) = 100\%$ 95% CI = 93.02% - 100%	Living Donor Plasma (EDTA) $76/(76+0) = 100\%$ 95% CI = 95.26% - 100%
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Negative Agreement

Using the data from the composite performance results above the negative agreement of the **ASI Evolution** can be calculated:

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Using this formula, the negative agreement is calculated as:

Cadaveric Donor Serum (Red Top) $74/(74+0) = 100\%$ 95% CI = 95.14% - 100%	Cadaveric Donor Serum (SST) $90/(90+0) = 100\%$ 95% CI = 95.98% - 100%	Cadaveric Donor Plasma (EDTA) $84/(84+0) = 100\%$ 95% CI = 95.70% - 100%
Living Donor Serum (Red Top) $75/(75+0) = 100\%$ 95% CI = 95.20% - 100%	Living Donor Serum (SST) $51/(51+0) = 100\%$ 95% CI = 93.02% - 100%	Living Donor Plasma (EDTA) $76/(76+0) = 100\%$ 95% CI = 95.26% - 100%

Sensitivity and Specificity

The sensitivity of the **ASI Evolution** using cadaveric and living donor samples can be calculated using the data from the performance results of the spiked samples. The sensitivity was determined by spiking the nonreactive specimens with one of five RPR reactive samples.

The specificity of the **ASI Evolution** using cadaveric and living donor samples can be calculated Using the data from the performance results of the nonreactive samples.

Serum – SST

Clinical Specificity (random donors)		Analytical Sensitivity (spiked samples)	
Cadaveric	Living	Cadaveric	Living
$90/90+0 = 100\%$	$51/51+0 = 100\%$	$90/90+0 = 100\%$	$51/51+0 = 100\%$
95% CI = 95.98 – 100%	95% CI = 93.02 – 100%	95% CI = 95.98 – 100%	95% CI = 93.02 – 100%

Serum – Red Top

Clinical Specificity (random donors)		Analytical Sensitivity (spiked samples)	
Cadaveric	Living	Cadaveric	Living
$74/74+0 = 100\%$	$75/75+0 = 100\%$	$74/74+0 = 100\%$	$75/75+0 = 100\%$
95% CI = 95.14 – 100%	95% CI = 95.2 – 100%	95% CI = 95.14 – 100%	95% CI = 95.2 – 100%

Plasma – EDTA

Clinical Specificity (random donors)		Analytical Sensitivity (spiked samples)	
Cadaveric	Living	Cadaveric	Living
$84/84+0 = 100\%$	$76/76+0 = 100\%$	$84/84+0 = 100\%$	$76/76+0 = 100\%$
95% CI = 95.7 – 100%	95% CI = 95.26 – 100%	95% CI = 95.7 – 100%	95% CI = 95.26 – 100%

Repeatability

Repeatability is defined as the variation in measurements taken by a single instrument on the same item and under the same conditions.

Repeatability testing was conducted at Arlington Scientific. The testing consisted of:

- Testing twenty (20) cadaveric samples prior to being spiked.
- Testing twenty (20) cadaveric samples spiked with a reactive specimen with a titer of 1:2 or 1:4.
- Testing twenty (20) living-donor samples prior to being spiked.
- Testing twenty (20) living-donor samples spiked with a reactive specimen with a titer of 1:2 or 1:4.
- Each sample was tested each day for six non-consecutive days by an operator with experience in performing the ASI RPR Test for Syphilis and operating the ASI Evolution.
- Each sample was tested with three different lots of carbon antigen.

The results of testing the cadaveric samples are contained in Tables below:

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					Results					
					ASI Evolution ID# 5800-1022					
	Sample ID	Sample Type	Hemolysis	Titer	7/1/2020	7/6/2020	7/8/2020	7/10/2020	7/13/2020	7/15/2020
Cadaveric Unspiked Samples – Carbon Antigen Lot CA9P02RRD										
1	30013647	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
2	30013615	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
3	30013646	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
4	30013663	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
5	10662892	Red Top	Moderate	N/A	NR	NR	NR	NR	NR	NR
6	10588533	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
7	20060904	Red Top	Moderate	N/A	NR	NR	NR	NR	NR	NR
8	10682077	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
9	10697670	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
10	10638866	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
11	10697669(2)	SST	None	N/A	NR	NR	NR	NR	NR	NR
12	10700530(2)	SST	Light	N/A	NR	NR	NR	NR	NR	NR
13	10670779(2)	SST	Heavy	N/A	NR	NR	NR	NR	NR	NR
14	10638858	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
15	10670781(2)	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
16	10619513	EDTA	Moderate	N/A	NR	NR	NR	NR	NR	NR
17	10638862	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
18	10670783	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
19	10585950	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
20	10577232(2)	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
Cadaveric Spiked Samples – Carbon Antigen Lot CA9P02RRD										
1	30013647	Red Top	Heavy	1:4	R	R	R	R	R	R
2	30013615	Red Top	None	1:2	R	R	R	R	R	R
3	30013646	Red Top	Light	1:4	R	R	R	R	R	R
4	30013663	Red Top	Heavy	1:4	R	R	R	R	R	R
5	10662892	Red Top	Moderate	1:2	R	R	R	R	R	R
6	10588533	Red Top	Heavy	1:2	R	R	R	R	R	R
7	20060904	Red Top	Moderate	1:2	R	R	R	R	R	R
8	10682077	SST	Moderate	1:4	R	R	R	R	R	R
9	10697670	SST	Moderate	1:2	R	R	R	R	R	R
10	10638866	SST	Moderate	1:2	R	R	R	R	R	R
11	10697669(2)	SST	None	1:4	R	R	R	R	R	R
12	10700530(2)	SST	Light	1:2	R	R	R	R	R	R
13	10670779(2)	SST	Heavy	1:4	R	R	R	R	R	R
14	10638858	EDTA	Light	1:2	R	R	R	R	R	R
15	10670781(2)	EDTA	Heavy	1:2	R	R	R	R	R	R
16	10619513	EDTA	Moderate	1:4	R	R	R	R	R	R
17	10638862	EDTA	Heavy	1:2	R	R	R	R	R	R
18	10670783	EDTA	Light	1:4	R	R	R	R	R	R
19	10585950	EDTA	Heavy	1:2	R	R	R	R	R	R
20	10577232(2)	EDTA	Light	1:4	R	R	R	R	R	R

					Results					
					ASI Evolution ID# 5800-1022					
	Sample ID	Sample Type	Hemolysis	Titer	7/1/2020	7/6/2020	7/8/2020	7/10/2020	7/13/2020	7/15/2020
Cadaveric Unspiked Samples – Carbon Antigen Lot CA9P19RA										
1	30013647	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
2	30013615	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
3	30013646	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
4	30013663	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
5	10662892	Red Top	Moderate	N/A	NR	NR	NR	NR	NR	NR
6	10588533	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
7	20060904	Red Top	Moderate	N/A	NR	NR	NR	NR	NR	NR
8	10682077	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
9	10697670	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
10	10638866	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
11	10697669(2)	SST	None	N/A	NR	NR	NR	NR	NR	NR
12	10700530(2)	SST	Light	N/A	NR	NR	NR	NR	NR	NR
13	10670779(2)	SST	Heavy	N/A	NR	NR	NR	NR	NR	NR
14	10638858	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
15	10670781(2)	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
16	10619513	EDTA	Moderate	N/A	NR	NR	NR	NR	NR	NR
17	10638862	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
18	10670783	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
19	10585950	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
20	10577232(2)	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
Cadaveric Spiked Samples – Carbon Antigen Lot CA9P18RA										
1	30013647	Red Top	Heavy	1:4	R	R	R	R	R	R
2	30013615	Red Top	None	1:2	R	R	R	R	R	R

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3	30013646	Red Top	Light	1:4	R	R	R	R	R	R
4	30013663	Red Top	Heavy	1:4	R	R	R	R	R	R
5	10662892	Red Top	Moderate	1:2	R	R	R	R	R	R
6	10588533	Red Top	Heavy	1:2	R	R	R	R	R	R
7	20060904	Red Top	Moderate	1:2	R	R	R	R	R	R
8	10682077	SST	Moderate	1:4	R	R	R	R	R	R
9	10697670	SST	Moderate	1:2	R	R	R	R	R	R
10	10638866	SST	Moderate	1:2	R	R	R	R	R	R
11	10697669(2)	SST	None	1:4	R	R	R	R	R	R
12	10700530(2)	SST	Light	1:2	R	R	R	R	R	R
13	10670779(2)	SST	Heavy	1:4	R	R	R	R	R	R
14	10638858	EDTA	Light	1:2	R	R	R	R	R	R
15	10670781(2)	EDTA	Heavy	1:2	R	R	R	R	R	R
16	10619513	EDTA	Moderate	1:4	R	R	R	R	R	R
17	10638862	EDTA	Heavy	1:2	R	R	R	R	R	R
18	10670783	EDTA	Light	1:4	R	R	R	R	R	R
19	10585950	EDTA	Heavy	1:2	R	R	R	R	R	R
20	10577232(2)	EDTA	Light	1:4	R	R	R	R	R	R

					Results					
					ASI Evolution ID# 5800-1022					
	Sample ID	Sample Type	Hemolysis	Titer	7/1/2020	7/6/2020	7/8/2020	7/10/2020	7/13/2020	7/15/2020
Cadaveric Unspiked Samples – Carbon Antigen Lot CA0A03RZZ										
1	30013647	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
2	30013615	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
3	30013646	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
4	30013663	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
5	10662892	Red Top	Moderate	N/A	NR	NR	NR	NR	NR	NR
6	10588533	Red Top	Heavy	N/A	NR	NR	NR	NR	NR	NR
7	20060904	Red Top	Moderate	N/A	NR	NR	NR	NR	NR	NR
8	10682077	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
9	10697670	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
10	10638866	SST	Moderate	N/A	NR	NR	NR	NR	NR	NR
11	10697669(2)	SST	None	N/A	NR	NR	NR	NR	NR	NR
12	10700530(2)	SST	Light	N/A	NR	NR	NR	NR	NR	NR
13	10670779(2)	SST	Heavy	N/A	NR	NR	NR	NR	NR	NR
14	10638858	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
15	10670781(2)	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
16	10619513	EDTA	Moderate	N/A	NR	NR	NR	NR	NR	NR
17	10638862	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
18	10670783	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
19	10585950	EDTA	Heavy	N/A	NR	NR	NR	NR	NR	NR
20	10577232(2)	EDTA	Light	N/A	NR	NR	NR	NR	NR	NR
Cadaveric Spiked Samples – Carbon Antigen Lot CA0A03RZZ										
1	30013647	Red Top	Heavy	1:4	R	R	R	R	R	R
2	30013615	Red Top	None	1:2	R	R	R	R	R	R
3	30013646	Red Top	Light	1:4	R	R	R	R	R	R
4	30013663	Red Top	Heavy	1:4	R	R	R	R	R	R
5	10662892	Red Top	Moderate	1:2	R	R	R	R	R	R
6	10588533	Red Top	Heavy	1:2	R	R	R	R	R	R
7	20060904	Red Top	Moderate	1:2	R	R	R	R	R	R
8	10682077	SST	Moderate	1:4	R	R	R	R	R	R
9	10697670	SST	Moderate	1:2	R	R	R	R	R	R
10	10638866	SST	Moderate	1:2	R	R	R	R	R	R
11	10697669(2)	SST	None	1:4	R	R	R	R	R	R
12	10700530(2)	SST	Light	1:2	R	R	R	R	R	R
13	10670779(2)	SST	Heavy	1:4	R	R	R	R	R	R
14	10638858	EDTA	Light	1:2	R	R	R	R	R	R
15	10670781(2)	EDTA	Heavy	1:2	R	R	R	R	R	R
16	10619513	EDTA	Moderate	1:4	R	R	R	R	R	R
17	10638862	EDTA	Heavy	1:2	R	R	R	R	R	R
18	10670783	EDTA	Light	1:4	R	R	R	R	R	R
19	10585950	EDTA	Heavy	1:2	R	R	R	R	R	R
20	10577232(2)	EDTA	Light	1:4	R	R	R	R	R	R

The results of testing the living-donor samples are contained in Tables below:

					Results					
					ASI Evolution ID# 5800-1022					
	Sample ID	Sample Type	Hemolysis	Titer	7/1/2020	7/6/2020	7/8/2020	7/10/2020	7/13/2020	7/15/2020
Living Unspiked Samples – Carbon Antigen Lot CA9P02RRD										
1	10662622	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
2	30033734	Red Top	None	N/A	NR	NR	NR	NR	NR	NR

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3	10667443	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
4	10682793	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
5	10680285	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
6	10682493	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
7	10683027	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
8	10542608	SST	None	N/A	NR	NR	NR	NR	NR	NR
9	10542614	SST	None	N/A	NR	NR	NR	NR	NR	NR
10	10615078	SST	Light	N/A	NR	NR	NR	NR	NR	NR
11	10542606	SST	None	N/A	NR	NR	NR	NR	NR	NR
12	10671043	SST	None	N/A	NR	NR	NR	NR	NR	NR
13	10542603	SST	None	N/A	NR	NR	NR	NR	NR	NR
14	10528444	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
15	10579337(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
16	10683025(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
17	10680289(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
18	10681600(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
19	10662622(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
20	10667443(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
Living Spiked Samples – Carbon Antigen Lot CA9P02RRD										
1	10662622	Red Top	None	1:4	R	R	R	R	R	R
2	30033734	Red Top	None	1:2	R	R	R	R	R	R
3	10667443	Red Top	Light	1:2	R	R	R	R	R	R
4	10682793	Red Top	None	1:4	R	R	R	R	R	R
5	10680285	Red Top	None	1:2	R	R	R	R	R	R
6	10682493	Red Top	None	1:4	R	R	R	R	R	R
7	10683027	Red Top	Light	1:2	R	R	R	R	R	R
8	10542608	SST	None	1:2	R	R	R	R	R	R
9	10542614	SST	None	1:2	R	R	R	R	R	R
10	10615078	SST	Light	1:4	R	R	R	R	R	R
11	10542606	SST	None	1:4	R	R	R	R	R	R
12	10671043	SST	None	1:2	R	R	R	R	R	R
13	10542603	SST	None	1:4	R	R	R	R	R	R
14	10528444	EDTA	None	1:4	R	R	R	R	R	R
15	10579337(2)	EDTA	None	1:2	R	R	R	R	R	R
16	10683025(2)	EDTA	None	1:4	R	R	R	R	R	R
17	10680289(2)	EDTA	None	1:2	R	R	R	R	R	R
18	10681600(2)	EDTA	None	1:4	R	R	R	R	R	R
19	10662622(2)	EDTA	None	1:2	R	R	R	R	R	R
20	10667443(2)	EDTA	None	1:2	R	R	R	R	R	R

Results										
ASI Evolution ID# 5800-1022										
Sample ID	Sample Type	Hemolysis	Titer	7/1/2020	7/6/2020	7/8/2020	7/10/2020	7/13/2020	7/15/2020	
Living Unspiked Samples – Carbon Antigen Lot CA9P18RA										
1	10662622	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
2	30033734	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
3	10667443	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
4	10682793	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
5	10680285	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
6	10682493	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
7	10683027	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
8	10542608	SST	None	N/A	NR	NR	NR	NR	NR	NR
9	10542614	SST	None	N/A	NR	NR	NR	NR	NR	NR
10	10615078	SST	Light	N/A	NR	NR	NR	NR	NR	NR
11	10542606	SST	None	N/A	NR	NR	NR	NR	NR	NR
12	10671043	SST	None	N/A	NR	NR	NR	NR	NR	NR
13	10542603	SST	None	N/A	NR	NR	NR	NR	NR	NR
14	10528444	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
15	10579337(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
16	10683025(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
17	10680289(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
18	10681600(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
19	10662622(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
20	10667443(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
Living Spiked Samples – Carbon Antigen Lot CA9P18RA										
1	10662622	Red Top	None	1:4	R	R	R	R	R	R
2	30033734	Red Top	None	1:2	R	R	R	R	R	R
3	10667443	Red Top	Light	1:2	R	R	R	R	R	R
4	10682793	Red Top	None	1:4	R	R	R	R	R	R
5	10680285	Red Top	None	1:2	R	R	R	R	R	R
6	10682493	Red Top	None	1:4	R	R	R	R	R	R
7	10683027	Red Top	Light	1:2	R	R	R	R	R	R
8	10542608	SST	None	1:2	R	R	R	R	R	R
9	10542614	SST	None	1:2	R	R	R	R	R	R
10	10615078	SST	Light	1:4	R	R	R	R	R	R

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11	10542606	SST	None	1:4	R	R	R	R	R	R
12	10671043	SST	None	1:2	R	R	R	R	R	R
13	10542603	SST	None	1:4	R	R	R	R	R	R
14	10528444	EDTA	None	1:4	R	R	R	R	R	R
15	10579337(2)	EDTA	None	1:2	R	R	R	R	R	R
16	10683025(2)	EDTA	None	1:4	R	R	R	R	R	R
17	10680289(2)	EDTA	None	1:2	R	R	R	R	R	R
18	10681600(2)	EDTA	None	1:4	R	R	R	R	R	R
19	10662622(2)	EDTA	None	1:2	R	R	R	R	R	R
20	10667443(2)	EDTA	None	1:2	R	R	R	R	R	R

					Results					
					ASI Evolution ID# 5800-1022					
	Sample ID	Sample Type	Hemolysis	Titer	7/1/2020	7/6/2020	7/8/2020	7/10/2020	7/13/2020	7/15/2020
Living Unspiked Samples – Carbon Antigen Lot CA0A03RZZ										
1	10662622	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
2	30033734	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
3	10667443	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
4	10682793	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
5	10680285	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
6	10682493	Red Top	None	N/A	NR	NR	NR	NR	NR	NR
7	10683027	Red Top	Light	N/A	NR	NR	NR	NR	NR	NR
8	10542608	SST	None	N/A	NR	NR	NR	NR	NR	NR
9	10542614	SST	None	N/A	NR	NR	NR	NR	NR	NR
10	10615078	SST	Light	N/A	NR	NR	NR	NR	NR	NR
11	10542606	SST	None	N/A	NR	NR	NR	NR	NR	NR
12	10671043	SST	None	N/A	NR	NR	NR	NR	NR	NR
13	10542603	SST	None	N/A	NR	NR	NR	NR	NR	NR
14	10528444	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
15	10579337(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
16	10683025(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
17	10680289(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
18	10681600(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
19	10662622(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
20	10667443(2)	EDTA	None	N/A	NR	NR	NR	NR	NR	NR
Living Spiked Samples – Carbon Antigen Lot CA0A03RZZ										
1	10662622	Red Top	None	1:4	R	R	R	R	R	R
2	30033734	Red Top	None	1:2	R	R	R	R	R	R
3	10667443	Red Top	Light	1:2	R	R	R	R	R	R
4	10682793	Red Top	None	1:4	R	R	R	R	R	R
5	10680285	Red Top	None	1:2	R	R	R	R	R	R
6	10682493	Red Top	None	1:4	R	R	R	R	R	R
7	10683027	Red Top	Light	1:2	R	R	R	R	R	R
8	10542608	SST	None	1:2	R	R	R	R	R	R
9	10542614	SST	None	1:2	R	R	R	R	R	R
10	10615078	SST	Light	1:4	R	R	R	R	R	R
11	10542606	SST	None	1:4	R	R	R	R	R	R
12	10671043	SST	None	1:2	R	R	R	R	R	R
13	10542603	SST	None	1:4	R	R	R	R	R	R
14	10528444	EDTA	None	1:4	R	R	R	R	R	R
15	10579337(2)	EDTA	None	1:2	R	R	R	R	R	R
16	10683025(2)	EDTA	None	1:4	R	R	R	R	R	R
17	10680289(2)	EDTA	None	1:2	R	R	R	R	R	R
18	10681600(2)	EDTA	None	1:4	R	R	R	R	R	R
19	10662622(2)	EDTA	None	1:2	R	R	R	R	R	R
20	10667443(2)	EDTA	None	1:2	R	R	R	R	R	R

There was 100% agreement between the results of all testing.

Using the Fisher Exact Test, a p-value can be calculated comparing cadaveric and living donor samples.

Repeatability Results

ASI Evolution Results for Cadaveric Donor Samples				
		Reactive	Nonreactive	Total
ASI Evolution Results for Living Donor Samples	Reactive	20	0	20
	Nonreactive	0	20	20
	Total	20	20	40

The Fisher exact statistic value is <0.00001. The result is significant at p <0.05.

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The data shows a very high degree of repeatability.

Reproducibility

Reproducibility is defined as the variation in measurements taken by multiple instruments on the same item and under the same conditions.

The interpretation of 20 samples using the ASI RPR Test for Syphilis and the ASI Evolution were evaluated for reactivity. The testing requirements were as follows:

1. Three different ASI Evolutions with the new algorithm were used for testing.
2. Ten cadaveric and ten living donor samples were used.
3. Each sample was tested 19 times on each instrument.
4. The same samples and reagents were used on all three instruments.

A total of 20 samples were evaluated to determine reproducibility of reactivity between the three instruments. Of the 20 samples, 14 were reactive and 6 were nonreactive. The reactive samples had titers of 1:2 (8 samples), 1:4 (2 sample), 1:8 (2 sample) and 1:16 (2 sample). Each of the 20 samples was repeated 19 times to evaluate the reactivity using the ASI Evolution. An aliquot of the same sample was dispensed into 19 tubes. All 19 tubes were placed into the ASI Evolution and the run was performed.

	Sample	Sample Type	Hemolysis	Expected	Results						% Agreement
					Titer	Evolution 1	Evolution 2	Evolution 3			
Cadaveric Donor Samples											
1	20071005	Red Top	Light	1:2	19/19	R	19/19	R	19/19	R	100%
2	20070804	Red Top	Moderate	1:8	19/19	R	19/19	R	19/19	R	100%
3	20070705	Red Top	Moderate	1:4	19/19	R	19/19	R	19/19	R	100%
4	20070806	Red Top	Light	1:2	19/19	R	19/19	R	19/19	R	100%
5	20070504	Red Top	Light	1:2	19/19	R	19/19	R	19/19	R	100%
6	20062806	Red Top	Light	1:2	19/19	R	19/19	R	19/19	R	100%
7	20061302	Red Top	Moderate	1:16	19/19	R	19/19	R	19/19	R	100%
8	10576054	Red Top	Heavy	NR	19/19	NR	19/19	NR	19/19	NR	100%
9	20060901	Red Top	Light	NR	19/19	NR	19/19	NR	19/19	NR	100%
10	20060806	Red Top	Moderate	NR	19/19	NR	19/19	NR	19/19	NR	100%
Living Donor Samples											
1	20060604	Red Top	None	1:16	19/19	R	19/19	R	19/19	R	100%
2	100558702(2)	Red Top	Light	1:8	19/19	R	19/19	R	19/19	R	100%
3	10682291	Red Top	None	1:2	19/19	R	19/19	R	19/19	R	100%
4	10663420	Red Top	None	1:4	19/19	R	19/19	R	19/19	R	100%
5	10681685	Red Top	None	1:2	19/19	R	19/19	R	19/19	R	100%
6	10630627	Red Top	None	1:2	19/19	R	19/19	R	19/19	R	100%
7	10666753	Red Top	None	1:2	19/19	R	19/19	R	19/19	R	100%
8	10703825	Red Top	Light	NR	19/19	NR	19/19	NR	19/19	NR	100%
9	10668273	Red	None	NR	19/19	NR	19/19	NR	19/19	NR	100%

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		Top Red Top									
10	10683282		Moderate	NR	19/19	NR	19/19	NR	19/19	NR	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.

15 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.
3. Jurado RL, Campbell J, Martin PD. 1993. *Arch Intern Med*, **153**:1496-1498.

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