Alinity i Syphilis TP (Syphilis)-21

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i Syphilis TP assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of antibodies (IgG and IgM) directed against *Treponema pallidum* (TP) in human serum and plasma on the Alinity i analyzer.

The Alinity i Syphilis TP assay is to be used as an **initial diagnostic test or** in conjunction with **a nontreponemal laboratory** test and clinical findings to aid in the diagnosis of syphilis infection.

Warning: The Alinity i Syphilis TP assay is not intended for use in screening blood, plasma, or tissue donors. The effectiveness of the Alinity i Syphilis TP assay for use in screening blood, plasma, or tissue donors has not been established.

SUMMARY AND EXPLANATION OF THE TEST

Syphilis is caused by infection with the bacterium *Treponema pallidum* (TP),[1] which is transmitted primarily by sexual contact or congenitally. The disease can present in different clinically defined stages (primary, secondary, latent, and tertiary); the latent phase is asymptomatic.

Upon infection with TP, an immune response develops that is directed not only against antigens specific to TP but also antigens released during the TP-mediated cellular damage. **Two types** of **tests** have been developed as aids to diagnose syphilis, **treponemal and nontreponemal**. A positive treponemal test result is an indication for an acute, latent, or past infection with TP. **Nontreponemal tests** are especially valuable for **monitoring** disease activity and therapy response.[2] It is common practice that reactive test results of either a treponemal or nontreponemal test are confirmed by a test of the complementary test type to enhance diagnostic accuracy.[2, 3]

The Alinity i Syphilis TP assay is a treponemal test that detects IgG and IgM antibodies to TP. Two different algorithms, which combine a treponemal with a nontreponemal test, are used as an aid in the diagnosis of syphilis. The algorithm starting with the treponemal test is called reverse screening algorithm and has been implemented in laboratories due to the availability of automated treponemal tests.[4]

Samples **reactive** in a treponemal test are **subjected to a nontreponemal supplementary test (e.g., Rapid Plasma Reagin [RPR]).**[3] The reverse screening algorithm for syphilis testing can identify persons previously treated for syphilis and those with untreated or incompletely treated syphilis.[3] **Discordant treponemal**-reactive, RPR-nonreactive results should be reflexed to a second treponemal test (e.g., Treponema Pallidum Particle Agglutination [TP-PA]) for further evaluation.[3]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the qualitative detection of antibodies (IgG and IgM) directed against TP in human serum or plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, recombinant TP antigen (TpN15, TpN17, and TpN47) coated paramagnetic microparticles, and assay diluent are combined and incubated. The Anti-TP antibodies present in the sample bind to the TP coated microparticles. The mixture is washed. Anti-human IgG and IgM acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of anti-TP in the sample and the RLUs detected by the system optics.

The presence or absence of anti-TP in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.

If the chemiluminescent signal in the reaction is greater than or equal to the cutoff signal, the specimen is considered reactive for anti-TP antibodies.

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity i Syphilis TP Reagent Kit 07P60

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	07P6021	07P6031
Tests per cartridge	100	600
Number of cartridges per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	4.2 mL	16.8 mL
CONJUGATE	4.2 mL	16.3 mL
ASSAY DILUENT	5.9 mL	28.7 mL

MICROPARTICLES TP (*E.coli*, recombinant) antigen coated microparticles in HEPES buffer with detergent. Minimum concentration: 0.08% solids. Preservatives: sodium azide and other antimicrobial agents.

CONJUGATE Murine anti-IgG/anti-IgM acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: (anti-IgG) 26.6 ng/mL / (anti-IgM) 1.34 ng/mL. Preservatives: sodium azide and other antimicrobial agents.

ASSAY DILUENT MES buffer with detergent. Preservatives: ProClin 950 and other antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[5], [6], [7], [8]

The following warnings and precautions apply to: MICROPARTICLES and CONJUGATE	
	
WARNING	Contain Polyethylene glycol octylphenyl ether (Triton X-405) and Sodium azide.
H319	Causes serious eye irritation.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: ASSAY DILUENT	
	
WARNING	Contains Polyethylene glycol octylphenyl ether (Triton X-100) and Methylisothiazolone.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice / attention.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

For the most current hazard information, see the product Safety Data Sheet.

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.

- Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.

- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.
Onboard	System Temperature	30 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity i Syphilis TP assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters**, refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, **refer to the Alinity ci-series Operations Manual.**

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Dipotassium EDTA
	Tripotassium EDTA
	Lithium heparin plasma separator
	Lithium heparin

Specimen Types	Collection Tubes
	Sodium heparin

Specimen Conditions

Do not use:

- heat-inactivated specimens
- grossly hemolyzed specimens
- specimens with obvious microbial contamination

- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter
- they require repeat testing.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low-speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at a minimum of 100 000 g-minutes.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below.

  Centrifugation time using alternate RCF values can be calculated using the following formula:

$$\text{Minimum Centrifugation time (minutes)} = \frac{100\,000 \text{ g-minutes}}{\text{RCF}}$$

Recentrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	10 000	100 000
20	5000	100 000
40	2500	100 000

$RCF = 1.12 \times r_{max} (rpm/1000)^2$

RCF - The relative centrifugal force generated during centrifugation.

rpm - The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).

Centrifugation Time - The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.

r_{max} - Radius of the rotor in millimeters. NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (r_{max}) should be manually measured in millimeters and the RCF calculated.

g-minutes - The unit of measure for the product of RCF (× g) and centrifugation time (minutes).

· Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature	72 hours	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8°C	7 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	-10°C or colder	30 days	Remove serum or plasma from the clot, red blood cells, or separator gel.

No qualitative performance differences were observed between experimental controls and 19 nonreactive or 17 spiked reactive specimens subjected to 6 freeze/thaw cycles; however, multiple freeze/thaw cycles should be avoided.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

07P60 Alinity i Syphilis TP Reagent Kit

Materials Required but not Provided

- Alinity i Syphilis TP assay file
- 07P6001 Alinity i Syphilis TP Calibrator
- 07P6010 Alinity i Syphilis TP Controls or other control material
- Alinity Pre-Trigger Solution
- Alinity Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

Assay Procedure

For a detailed description of how to run an assay, **refer to the Alinity ci-series Operations Manual, Section 5.**

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

Priority:

- Sample volume for first test: 80 µL
- Sample volume for each additional test from same sample cup: 30 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 150 µL
- Sample volume for each additional test from same sample cup: 30 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i Syphilis TP calibrator package insert and/or Alinity i control package insert for preparation and usage.
- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, **Section 9. Perform maintenance more frequently when required by laboratory procedures.**

Sample Dilution Procedures

Samples cannot be diluted for the Alinity i Syphilis TP assay.

Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.

Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

- **If statistically-based quality control limits are not available**, then the calibration should **not exceed a 30-day limit for recalibration frequency**.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

The recommended control requirement for the Alinity i Syphilis TP assay is that a single sample of each control level be tested once every day testing performed.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by **assaying a minimum of 20 replicates over several (3-5) days** and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations

- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.[9]

- If quality control results do not meet the acceptance criteria defined by laboratory QC procedure, sample results may be suspect. Follow the established quality control procedures to troubleshoot. Recalibration may be necessary. **For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[10]

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity i analyzer calculates results for the Alinity i Syphilis TP assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.

Cutoff RLU = Calibrator 1 Mean RLU x 0.20

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

The cutoff is 1.00 S/CO.

Result (S/CO)	Instrument Result	Interpretation
< 1.00	Nonreactive	Nonreactive for treponemal antibodies
≥ 1.00	Reactive	Reactive for treponemal antibodies

Test results are intended to aid in diagnosis only. As with all serological tests for syphilis, **results should always be interpreted in conjunction with additional treponemal or nontreponemal serologic test results (as appropriate), the patient's clinical symptoms, medical history, and other clinical and/or laboratory findings to produce a diagnosis of syphilis by disease stage.**

Diagnostic considerations should be based on treponemal and nontreponemal testing as described in the Centers for Disease Control and Prevention (CDC) Sexually Transmitted Diseases Treatment Guidelines, 2015.[3]

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- If the Alinity i Syphilis TP results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- **A reactive test** result for treponemal antibodies **is not diagnostic** of syphilis without **additional** serologic testing and a full clinical evaluation.[3]
- False reactive results can be expected with any test kit. The proportion of these falsely reactive specimens is dependent upon the specificity of the test kit, specimen integrity, and the characteristics of the local population being screened.
- **A nonreactive** treponemal test result **does not exclude** the possibility of exposure to or infection with syphilis. **Antibodies** may be at **low or undetectable** levels in incubating or **early primary** disease and in some clinical conditions.[3]
- Results in samples from immunosuppressed patients or from patients with disorders leading to immunosuppression should be interpreted with caution.
- The **detection of treponemal antibodies** may **indicate recent, past,** or **successfully treated** syphilis. This test cannot **distinguish between active and treated infection**, and therefore may not be used to determine response to therapy, relapse, or reinfection.[3]
- Assay interference due to circulating antibodies against yaws, pinta, and bejel has not been evaluated. Cross-reactivity with these treponemal disease conditions is to be expected.

EXPECTED VALUES

This study was performed on the ARCHITECT i System.

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

A total of 1145 specimens prospectively collected from the intended use population were tested using the ARCHITECT Syphilis TP assay; 673 (58.8%) were female and 472 (41.2%) were male. The mean age was 35 years (age range: 6 to 91 years).

The ARCHITECT Syphilis TP assay was reactive in 163 (14.2%) of the prospectively collected specimens in the intended use population. Testing of the specimens was performed at three clinical testing sites located in San Antonio, Texas; Baltimore, Maryland; and Temple, Texas.

The distribution of ARCHITECT Syphilis TP reactive and nonreactive results by age and gender is summarized in the following table.

Age Range (Years)	Gender	ARCHITECT Syphilis TP Result		Total
		Number of Reactive (%)	Number of Nonreactive (%)	
2 to 12	Female	0 (0.0)	1 (100.0)	1
13 to 21	Female	1 (0.8)	118 (99.2)	119
	Male	1 (6.3)	15 (93.8)	16
22 to 29	Female	6 (2.6)	229 (97.4)	235
	Male	12 (23.5)	39 (76.5)	51
30 to 39	Female	6 (2.9)	199 (97.1)	205
	Male	32 (23.7)	103 (76.3)	135
40 to 49	Female	9 (12.9)	61 (87.1)	70
	Male	50 (29.6)	119 (70.4)	169
50 to 59	Female	5 (21.7)	18 (78.3)	23
	Male	29 (40.8)	42 (59.2)	71
60 to 64	Female	3 (50.0)	3 (50.0)	6
	Male	2 (16.7)	10 (83.3)	12
65 to 100	Female	1 (7.1)	13 (92.9)	14
	Male	6 (33.3)	12 (66.7)	18
	Total	163 (14.2)	982 (85.8)	1145

The 1145 prospectively-collected specimens from the intended use population included 442 specimens sent for routine syphilis testing (325 female and 117 male, 6–91 years old), 304 pregnant females (16–43 years old), and 399 human immunodeficiency virus (HIV) positive individuals (44 female and 355 male, 18–72 years old).

The ARCHITECT Syphilis TP results for each category in the intended use population are summarized in the following table.

Category	ARCHITECT Syphilis TP Result

	Number of Reactive (%)	Number of Nonreactive (%)	Total
Routine Syphilis	38 (8.6%)	404 (91.4%)	442
Pregnant	1 (0.3%)	303 (99.7%)	304
HIV Positive	124 (31.1%)	275 (68.9%)	399
Total	163 (14.2%)	982 (85.8%)	1145

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2.*11* Testing was conducted using 3 lots of the Alinity i Syphilis TP Reagent Kit, 3 lots of the Alinity i Syphilis TP Calibrator, and 3 lots of the Alinity i Syphilis TP Controls and 1 instrument. Two controls and 4 recalcified human plasma panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

			Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	n	Mean (S/CO)	SD	%CV	SD (Range[b])	%CV (Range[b])
Negative Control	359	0.03	0.003	9.9	0.004 (0.002-0.005)	12.1 (7.7-14.6)
Positive Control	355[c]	2.65	0.067	2.5	0.117 (0.109-0.127)	4.4 (4.2-4.7)
Nonreactive Panel	360	0.08	0.007	8.7	0.007 (0.005-0.010)	9.5 (6.7-12.7)

Sample	n	Mean (S/CO)	Within-Run (Repeatability) SD	Within-Run (Repeatability) %CV	Within-Laboratory (Total)[a] SD (Range[b])	Within-Laboratory (Total)[a] %CV (Range[b])
High Nonreactive Panel	352	0.51	0.014	2.8	0.022 (0.021-0.023)	4.3 (3.9-4.6)
Low Reactive Panel	360	1.18	0.031	2.6	0.045 (0.043-0.048)	3.9 (3.7-4.1)
High Reactive Panel	352	3.38	0.149	4.4	0.180 (0.112-0.267)	5.3 (3.3-8.1)

[a]Includes within-run, between-run, and between-day variability.

[b]Maximum and minimum SD or %CV for each reagent lot and instrument combination.

[c] An outlying run was observed. Based on guidance from CLSI EP05-A2, a replacement run was performed and the results are shown in the table above. Without the replacement run, the within-run (repeatability) %CV was 5.6% and the within-laboratory precision (total) %CV was 6.4%.

System Reproducibility

This study was performed on the ARCHITECT i2000SR System.

A 5-day precision study was performed for the ARCHITECT Syphilis TP assay based on guidance from CLSI documents EP05-A2 and EP15-A2.*11*, *12* Testing was conducted at 3 clinical sites using 3 lots each of ARCHITECT Syphilis TP Reagents, 2 lots of ARCHITECT Syphilis TP Calibrator, and 1 lot of ARCHITECT Syphilis TP Controls, on 1 instrument per site. Two levels of controls and 4 serum panels were assayed in replicates of 4 at 2 separate times of day for 5 days.

Sample	N	Grand Mean S/CO	Within-Run		Within-Day		Within-Laboratory (Total)			Precision with Additional Component of Between-Site		Precision with Additional Component of Between-Lot		Precision with Additional Components of Site and Lot (Overall)	
			SD	%CV	SD	%CV	SD	%CV	95% CI	SD	%CV	SD	%CV	SD	%CV
Negative Control	360	0.03	0.002	6.4	0.002	6.6	0.002	6.6	(6.24,7.08)	0.003	9.3	0.003	9.5	0.003	9.6
Positive Control[a]	360	2.72	0.108	4.0	0.130	4.8	0.134	4.9	(4.59,5.35)	0.134	4.9	0.134	4.9	0.134	4.9
Nonreactive Panel	360	0.08	0.004	5.1	0.004	5.1	0.004	5.4	(5.07,5.78)	0.006	7.5	0.006	7.4	0.007	8.8
High Nonreactive Panel	360	0.55	0.012	2.3	0.015	2.7	0.015	2.8	(2.61,3.04)	0.017	3.2	0.017	3.2	0.017	3.2
Low Reactive Panel	360	1.24	0.023	1.9	0.027	2.2	0.029	2.4	(2.18,2.56)	0.034	2.7	0.035	2.8	0.035	2.8
High Reactive Panel	360	3.57	0.073	2.0	0.079	2.2	0.094	2.6	(2.43,2.91)	0.107	3.0	0.100	2.8	0.107	3.0

[a] An outlying run was observed at one site for one reagent lot. The total %CV for the Positive Control using the replacement run was 2.6%.

Percent Agreement

A study was performed to determine the percent agreement between the Alinity i Syphilis TP assay and the ARCHITECT Syphilis TP assay. Reactive and nonreactive Syphilis specimens were assayed across 2 lots of the Alinity i Syphilis Reagent Kit, 2 lots of the Alinity i Syphilis TP Calibrator, and 2 lots of the Alinity i Syphilis TP Controls and 2 instruments. The specimens were also tested on 2 ARCHITECT i2000SR instruments using 2 lots each of the ARCHITECT Syphilis TP Reagent Kit, ARCHITECT Syphilis TP Calibrator, and ARCHITECT Syphilis TP Controls. The percent agreement between the Alinity i Syphilis TP assay and the ARCHITECT Syphilis TP assay for reactive and nonreactive specimens is presented in the table below.

	ARCHITECT Syphilis TP	
Alinity i Syphilis TP	**Reactive**	**Nonreactive**
Reactive	455	1
Nonreactive	1	173

Positive % agreement = 99.78% (455/456); 95% Confidence Interval = 98.78% to 99.99%

Negative % agreement = 99.43% (173/174); 95% Confidence Interval = 96.84% to 99.99%

Clinical Performance

This study was performed on the ARCHITECT i2000SR System.

A multicenter study was conducted on the ARCHITECT i System to evaluate the ability of the ARCHITECT Syphilis TP assay to detect antibodies (IgG and IgM) directed against *Treponema pallidum* (TP).

A total of 2222 specimens were tested in the ARCHITECT Syphilis TP clinical study. Two specimens were excluded due to specimen issues. The remaining 2220 specimens included 1145 prospectively collected from the intended use population; 406 pre-selected positive for antibodies directed against TP based on previous laboratory testing (including 20 pregnant women known to be reactive for syphilis antibodies); 480 from apparently healthy individuals; 179 from medically diagnosed individuals with primary, secondary, or latent syphilis; and
10 specimens from pregnant females spiked with high antibody-positive syphilis TP specimens.

Each specimen prospectively collected from the intended use population or pre-selected positive for antibodies directed against TP based on previous laboratory testing was tested using the ARCHITECT Syphilis TP assay and the following comparator assays: a treponemal chemiluminescent immunoassay (TP-CLIA), a nontreponemal assay (Rapid Plasma Reagin [RPR]), and a second treponemal assay (Treponema Pallidum Particle Agglutination [TP-PA]). The final comparator result was determined using a 2 out of 3 rule (TP-CLIA, RPR, and TP-PA).

The clinical performance of the ARCHITECT Syphilis TP assay was evaluated by calculating positive percent agreement and negative percent agreement of the assay with the final comparator result.

During the clinical study (total number of tests = 2220), 2 exception codes prevented initial results and were retested to generate a final result.

Clinical Performance in Prospectively-Collected Specimens in Intended Use Population

Of the total 2220 specimens analyzed in the ARCHITECT Syphilis TP clinical study, 1145 were prospectively-collected intended use population specimens. These included 442 specimens sent for routine syphilis testing (325 female and 117 male, 6–91 years old), 304 pregnant females (16–43 years old), and 399 HIV positive (44 female and 355 male, 18–72 years old). Of the 1145 prospectively-collected specimens in the intended use population, 136 were pediatric specimens (6–21 years old).

The 1145 specimens included in the intended use population were prospectively sourced or collected at the following locations:

- Baltimore, Maryland: 12%
- Colton, California: 6%
- Fort Lauderdale, Florida: 8%
- Hyannis, Massachusetts: <1%
- Los Angeles, California: 20%
- Miami, Florida: 27%
- San Antonio, Texas: 12%
- Temple, Texas: 15%
- Location Unknown: <1%

A summary of the serological test profile for all prospectively-collected specimens in the intended use population is summarized in the following table.

TP-CLIA	RPR	TP-PA	Final Comparator Result	ARCHITECT	Number of Subjects
+	+	+	+	+	113
+	+	I	+	+	1
+	-	+	+	+	37
+	-	-	-	-	4
+	-	-	-	+	2
-	+	+	+	-	6
-	+	+	+	+	1

TP-CLIA	RPR	TP-PA	Final Comparator Result	ARCHITECT	Number of Subjects
-	+	-	-	-	167
-	+	-	-	+	3
-	+	I	-	-	1[a]
-	+	I	-	+	1[a]
-	-	+	-	-	8
-	-	+	-	+	2
-	-	-	-	-	796
-	-	-	-	+	2
E	-	+	+	+	1[b]
				Total	1145

+ = Positive/Reactive

- = Negative/Nonreactive

E = Equivocal

I = Inconclusive

[a] Two specimens that were TP-CLIA nonreactive, RPR reactive, and TP-PA inconclusive were assigned a final comparator result of negative based on the nonreactive treponemal test.

[b] One specimen that was TP-CLIA equivocal, RPR nonreactive, and TP-PA reactive was assigned a final comparator result of positive based on the reactive treponemal test.

Percent Agreement

The comparison between the ARCHITECT Syphilis TP result and the final comparator result for the prospectively-collected specimens in the intended use population is summarized in the following table.

ARCHITECT Syphilis TP Result Interpretation	Final Comparator Result	
	Reactive	Nonreactive
Reactive	153	10
Nonreactive	6[a]	976

[a] Six specimens were nonreactive by TP-CLIA and reactive by TP-PA and RPR.

Positive percent agreement was 96.2% (153/159) with a 95% confidence interval of 92.0% to 98.3%. Negative percent agreement was 99.0% (976/986) with a 95% confidence interval of 98.1% to 99.4%

Percent Agreement by Category

The percent agreement between the ARCHITECT Syphilis TP result and the final comparator result for each category of the prospectively-collected specimens in the intended use population is summarized in the following table.

Category	Positive Percent Agreement % (x/n)	95% Confidence Interval (%)	Negative Percent Agreement % (x/n)	95% Confidence Interval (%)
Routine Syphilis	97.3 (36/37)	86.2–99.5	99.5 (403/405)	98.2–99.9
Pregnant	NA	NA	99.7 (303/304)	98.2–99.9
HIV Positive	95.9 (117/122)	90.8–98.2	97.5 (270/277)	94.9–98.8

NA = not applicable

Clinical Performance in Pre-Selected Positive Specimens

Of the total 2220 specimens analyzed in the ARCHITECT Syphilis TP clinical study, 406 specimens were pre-selected positive for antibodies directed against TP based on previous laboratory testing (RPR and/or TP-PA). These included 386 presumed positive (106 female and 278 male, 16–78 years old). Gender was not reported for 2 specimens. Pre-selected positive specimens also included 20 reactive pregnant female specimens (17–36 years old). Of the
406 pre-selected positive specimens, 25 were pediatric specimens (16–21 years old).

A summary of the serological test profile for all pre-selected positive specimens is summarized in the following table.

TP-CLIA	RPR	TP-PA	Final Comparator Result	ARCHITECT	Number of Subjects
+	+	+	+	+	259
+	+	I	+	+	1
+	-	+	+	-	2
+	-	+	+	+	116
+	-	-	-	-	1
+	-	-	-	+	2
+	-	I	+	-	1[a]
-	+	-	-	-	3
-	-	-	-	-	20

TP-CLIA	RPR	TP-PA	Final Comparator Result	ARCHITECT	Number of Subjects
E	-	+	+	-	1[b]
				Total	406

+ = Positive/Reactive

- = Negative/Nonreactive

E = Equivocal

I = Inconclusive

[a] One specimen that was TP-CLIA reactive, RPR nonreactive, and TP-PA inconclusive was assigned a final comparator result of positive based on the reactive treponemal test.

[b] One specimen that was TP-CLIA equivocal, RPR nonreactive, and TP-PA reactive was assigned a final comparator result of positive based on the reactive treponemal test.

Percent Agreement

The comparison between the ARCHITECT Syphilis TP result and the final comparator result for all pre-selected positive specimens is summarized in the following table.

ARCHITECT Syphilis TP Result Interpretation	Final Comparator Result	
	Reactive	Nonreactive
Reactive	376	2
Nonreactive	4	24

Positive percent agreement was 98.9% (376/380) with a 95% confidence interval of 97.3% to 99.6%. Negative percent agreement was 92.3% (24/26) with a 95% confidence interval of 75.9% to 97.9%.

Percent Agreement by Category

The percent agreement between the ARCHITECT Syphilis TP result and the final comparator result for each category in the pre-selected positive population is summarized in the following table.

Category	Positive Percent Agreement % (x/n)	95% Confidence Interval (%)	Negative Percent Agreement % (x/n)	95% Confidence Interval (%)
Presumed Positive	98.9 (356/360)	97.2–99.6	92.3 (24/26)	75.9–97.9
Reactive Pregnant	100.0 (20/20)	83.9–100.0	NA	NA

NA = not applicable

Clinical Performance in Apparently Healthy Individuals

Of the total 2220 specimens analyzed in the ARCHITECT Syphilis TP clinical study, 480 specimens were from apparently healthy individuals. These included 244 females and 236 males, adults (22–89 years old), and pediatrics (0–21 years old).

	ARCHITECT Syphilis TP Result		
Category	**Number of Reactive (%)**	**Number of Nonreactive (%)**	**Total**
Adults	15 (4.1%)[a]	352 (95.9%)	367
Pediatrics	0 (0.0%)	113 (100.0%)	113
Total	15 (3.1%)	465 (96.9%)	480

[a] Fourteen specimens were reactive and 1 specimen was equivocal by TP-CLIA.

Clinical Performance in Medically Diagnosed Individuals

Of the total 2220 specimens analyzed in the ARCHITECT Syphilis TP clinical study, 179 were from individuals medically diagnosed with primary, secondary, or latent syphilis. Medical diagnosis was made by a licensed physician based on the patient's clinical information and the results of serological testing, such as a positive test for syphilis (Venereal Disease Research Laboratory [VDRL] test, RPR, and/or TP-PA) at the time the specimen was collected. These included 9 females and 170 males, adults (22– 66 years old), and pediatrics (18–21 years old).

Medically Diagnosed Individuals			**ARCHITECT Syphilis TP Result**	
Syphilis Stage	**Treatment Status**	**N**	**Reactive**	**Nonreactive**
Primary	Treated	44	33	11[a]
	Untreated	25	25	0
Secondary	Treated	29	29	0
	Untreated	27	27	0
Latent	Treated	25	25	0
	Untreated	29	29	0

[a] Nine specimens were nonreactive by TP-CLIA.

CLINICAL PERFORMANCE IN PREGNANT FEMALES

A total of 334 pregnant female specimens were analyzed in the ARCHITECT Syphilis TP clinical study. These included adults (22–43 years old) and pediatrics (16–21 years old). Age was not reported for 10 specimens.

The percent agreement for the prospectively-collected and the pre-selected positive specimens between the ARCHITECT Syphilis TP results and the final comparator results for the pregnant female population by trimester is summarized in the following table.

Category	Positive Percent Agreement % (x/n)	95% Confidence Interval (%)	Negative Percent Agreement % (x/n)	95% Confidence Interval (%)
Prospectively-Collected	NA	NA	99.7 (303/304)	98.2–99.9
First Trimester	NA	NA	100.0 (13/13)	77.2–100.0
Second Trimester [a]	NA	NA	99.2 (126/127)	95.7–99.9
Third Trimester	NA	NA	100.0 (161/161)	97.7–100.0
Trimester Unknown	NA	NA	100.0 (3/3)	43.8–100.0
Pre-Selected Positive	100.0 (20/20)	83.9–100.0	NA	NA
First Trimester	100.0 (1/1)	20.7–100.0	NA	NA
Second Trimester	NA	NA	NA	NA
Third Trimester	100.0 (5/5)	56.6–100.0	NA	NA
Trimester Unknown	100.0 (14/14)	78.5-100.0	NA	NA

NA = not applicable

[a] One specimen reactive on ARCHITECT was reactive by TP-CLIA and nonreactive by RPR and TP-PA.

In addition, 10 nonreactive pregnant female specimens that were spiked with high antibody-positive syphilis TP specimens were reactive by ARCHITECT Syphilis TP and TP CLIA. Positive percent agreement was 100.0% with a confidence interval of 72.2% to 100.0%.

Analytical Specificity

This study was performed on the ARCHITECT i2000SR System.

The ARCHITECT Syphilis TP assay was evaluated for potential cross-reactivity from specimens from individuals with medical conditions and other disease states unrelated to a syphilis infection.

Six specimens reactive in ARCHITECT Syphilis TP and a treponemal chemiluminescent immunoassay (TP-CLIA) were also either positive (marked by [a] in table below) or indeterminate (marked by [d] in table below) by confirmation testing. Confirmed reactive

results were not unexpected because the specimens had been obtained from vendors based on the required disease condition and documentation of prior syphilis laboratory test results was not provided by the vendor.

One of the 10 CMV IgG specimens, 1 of the 10 HIV specimens, 1 of the 7 monoclonal hyper IgG, and 1 of the 10 anti-*E. coli* specimens (marked by [b] in table below) were reactive in ARCHITECT Syphilis TP and were not confirmed with other treponemal or nontreponemal tests applied in this study.

One of the 11 gonorrhea specimens and 2 of the 6 HTLV-II specimens (marked by [c] in table below) were reactive in ARCHITECT Syphilis TP but could not be tested by the other tests (TP-CLIA, RPR, TP-PA, Fluorescent Treponemal Antibody Absorption [FTA ABS]) because specimens from these disease states could only be sourced as plasma specimens.

Clinical Category	N	Number of ARCHITECT Syphilis TP Reactive Results
Chlamydia	15	1[a]
Cytomegalovirus (CMV) IgG	10	1[b]
Cytomegalovirus (CMV) IgM	10	1[a]
Anti-dsDNA Autoantibodies	3	0
Epstein-Barr Virus (EBV) IgG	10	1[a]
Epstein-Barr Virus (EBV) IgM	24	0
Anti-Escherichia coli (*E. coli*)	10	1[b]
Gonorrhea	11	1[c]
HAVAB IgG	10	0
HBc IgM	4	0
Hemodialysis	10	0
Hepatitis A Virus (HAV)	10	1[a]
Hepatitis B Virus (HBV)	10	0
Hepatitis C Virus (HCV)	10	1[a]
Herpes Simplex Virus (HSV)	10	1[d]
Human Anti-Mouse Antibodies (HAMA)	10	0
Human Immunodeficiency Virus (HIV)	10	1[b]
Human T-Lymphotropic Virus-I (HTLV-I)	10	0
Human T-Lymphotropic Virus-II (HTLV-II)	6	2[c]
Influenza Vaccine Recipient	20	0
Leptospirosis	6	0
Leptospirosis IgM	5	0
Lyme Disease	10	0

Clinical Category	N	Number of ARCHITECT Syphilis TP Reactive Results
Monoclonal Hyper IgG	7	1[b]
Anti-Nuclear Antibody (ANA)	10	0
Polyclonal Hyper IgG	3	0
Pregnant	90	0
Rheumatoid Factor	10	0
Rubella IgG	10	0
Systemic Lupus Erythematosus	10	0
Toxoplasma gondii IgG	12	0
Toxoplasma gondii IgM	3	0
Transplant Recipient	10	0
Varicella Zoster Virus	10	0
Total	409	13

[a] Specimen was confirmed to be positive by other tests (TP-CLIA, RPR, TP-PA, FTA-ABS).

[b] Specimen was not confirmed positive by other tests (TP-CLIA, RPR, TP-PA, FTA-ABS).

[c] Specimen was reactive by ARCHITECT Syphilis TP but was not confirmed due to tube type limitations of the other tests (TP-CLIA, RPR, TP-PA, FTA-ABS).

[d] Specimen was positive by TP-CLIA and indeterminate by TP-PA and FTA-ABS.

Interference

This study was performed on the ARCHITECT i2000SR System.

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07-A2.*13* Potentially interfering substances were evaluated to determine whether S/CO values were affected when using the ARCHITECT Syphilis TP assay. Samples containing the potential interferents were prepared at 2 levels of syphilis (approximately 0.80 S/CO and 1.20 S/CO). The samples were assayed, and the S/CO values of the spiked samples were compared to the reference samples. The ARCHITECT Syphilis TP assay is not susceptible to interference effects from the following interferents at the interferent levels listed in the table below.

Interferent	Interferent Level
Conjugated Bilirubin	≤ 20 mg/dL
Unconjugated Bilirubin	≤ 20 mg/dL
Cholesterol	≤ 500 mg/dL
Gamma Globulin	≤ 6 g/dL
Hemoglobin	≤ 500 mg/dL

Interferent	Interferent Level
Triglycerides	≤ 3000 mg/dL
Total Protein	≤ 12 g/dL

Tube Type Matrix Comparison

This study was performed on the ARCHITECT i2000SR System.

The following tube types are acceptable for use with the ARCHITECT Syphilis TP assay:

- Serum, including serum separator
- Plasma: dipotassium EDTA, tripotassium EDTA, lithium heparin plasma separator, lithium heparin, and sodium heparin

The ARCHITECT Syphilis TP assay showed the following mean/median S/CO difference and distribution of S/CO difference for nonreactive samples when compared to the control tube type (serum).

	Nonreactive Samples (Unspiked)					High Nonreactive Samples (Target 0.80 S/CO)				
			Distribution of S/CO Differences					Distribution of S/CO Differences		
Tube Type	N	Mean/ Median S/CO Difference[a]	< 0.10 S/CO	0.10–0.20 S/CO	> 0.20 S/CO	N	Mean/ Median S/CO Difference[a]	< 0.10 S/CO	0.10–0.20 S/CO	> 0.20 S/CO
Serum Separator, Plastic	28	0.00	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	27	0.02	81.5% (22/27)	18.5% (5/27)	0.0% (0/27)
Dipotassium EDTA	28	-0.00	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	27	0.02	81.5% (22/27)	18.5% (5/27)	0.0% (0/27)
Tripotassium EDTA	27	-0.00	100.0% (27/27)	0.0% (0/27)	0.0% (0/27)	27	0.02	85.2% (23/27)	11.1% (3/27)	3.7% (1/27)
Lithium Heparin Plasma Separator	28	-0.01	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	27	0.01	88.9% (24/27)	11.1% (3/27)	0.0% (0/27)
Lithium Heparin	28	-0.01	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	27	0.02	88.9% (24/27)	7.4% (2/27)	3.7% (1/27)
Sodium Heparin	28	-0.01	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	26	0.00	88.5% (23/26)	11.5% (3/26)	0.0% (0/26)

[a] If the Shapiro-Wilk p-value is ≤ 0.0100, then the value displayed is the median.

The ARCHITECT Syphilis TP assay showed the following mean/median percent difference and distribution of percent difference for reactive samples when compared to the control tube type (serum).

	Low Reactive Samples (Target 1.20 S/CO)					High Reactive Samples (Target 6.00 S/CO)				
			Distribution of % Differences					Distribution of % Differences		
Tube Type	N	Mean/ Median Difference[a]	< 10%	10–20%	> 20 %	N	Mean/ Median % Difference[a]	< 10%	10–20%	> 20%
Serum Separator, Plastic	28	0.3	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	28	-0.1	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)
Dipotassium EDTA	28	0.9	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)	28	-0.7	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)
Tripotassium EDTA	28	-0.0	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	27	-0.3	100.0% (27/27)	0.0% (0/27)	0.0% (0/27)
Lithium Heparin Plasma Separator	27	3.7	63.0% (17/27)	33.3% (9/27)	3.7% (1/27)	28	-0.1	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)
Lithium Heparin	28	-0.3	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	28	-0.0	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)
Sodium Heparin	28	0.8	96.4% (27/28)	3.6% (1/28)	0.0% (0/28)	28	-0.3	100.0% (28/28)	0.0% (0/28)	0.0% (0/28)

[a] If the Shapiro-Wilk p-value is ≤ 0.0100, then the value displayed is the median.

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